ESSENTIAL AMINO ACID NUTRITIONAL ECOLOGY OF BOBWHITE QUAIL

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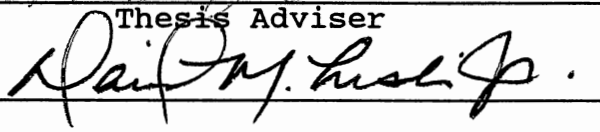

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CHAPTER I

INTRODUCTION

This thesis is composed of 4 manuscripts formatted for submission to selected scientific journals. Each manuscript is complete as written and does not require additional support material. The order of arrangement for each manuscript is text, literature cited, tables, and figures. Chapter II, "Relationship of serum and muscle free amino acids to dietary protein in bobwhite quail," is written in the format of the Auk. Chapter III, "Influence of season and brush management on nutritional condition and diet quality of bobwhite quail," is written in the format of the Journal of Wildlife Management. Chapter IV, "The use of crude protein to assess diet quality: a reexamination with quail," is written in the format of the American Naturalist. Chapter V, "Profiles of serum amino acids to assess condition of cotton rats (Sigmodon hispidus)," is written in the format of the Journal of Mammalogy.

CHAPTER II

RELATIONSHIP OF SERUM AND MUSCLE FREE AMINO ACIDS TO DIETARY PROTEIN IN BOBWHITE QUAIL

ABSTRACT.-- We investigated effects of dietary protein quality on the body condition of subadult and juvenile bobwhite quail (Colinus virginianus) and the use of serum and muscle free amino acids to asses the dietary protein status of juvenile quail. Thirty subadult and 36 juvenile quail were subjected to three experimental dietary protein treatments (8, 15, 33% protein). Morphological alterations in development were more pronounced, and more useful as indicators of protein nutritional condition, in younger aged chicks. Body weight was reduced in both subadult and juvenile quail subjected to the low dietary protein ration. Relative liver weight was a good indicator of protein malnutrition in both age groups. Total concentrations of branch chain amino acids (BCAA), and ratios of branch chain/non-branch chain amino acids (BCAA/NBCAA), essential amino acid/non-essential amino acid (EAA/NEAA), glycine/(leucine + valine), and serine/threonine in blood serum were excellent discriminators among diet groups. Likewise, concentrations of carnosine and taurine and ratios

of serine/threonine and glycine/(leucine + valine) were promising discriminators in muscle tissue homogenates. Clinical profiles of selected amino acids in serum and muscle can provide a useful technique for assessing protein nutritional status and diet quality of bobwhite quail.

Populations of bobwhite quail (Colinus virginianus) are known to fluctuate widely both within and between years due to changes in net productivity which is strongly dependent upon chick survival (Roseberry and Klimstra 1972; Cantu and Everett 1982; Servello and Kirkpatrick 1987). Chick mortality is often very high during the first few weeks of development and may be related to range condition (Wiseman and Lewis 1981; Cantu and Everett 1982; Webb and Guthery 1982) and nutrition (Hurst 1972). Several studies have indicated that survival of many upland game bird chicks is particularly dependent on the availability of protein (Moss 1973; White 1978; Beckerton and Middleton 1982; Wood et al. 1986).

Many useful techniques have been developed for assessing the overall nutritional status or health of bobwhite quail, including measures of body weight, body composition, selected organ and glandular development, and parasite burdens (Boorman and Lewis 1971; Robel 1972; Dabney and Dimmick 1977; Kirkpatrick 1980; Wood et al. 1986; Koerth and Guthery 1988). By far the most frequently used approach of assessing nutritional condition of quail has incorporated measures of lipid reserves such as total weight of

dissectable or extractable body fat, gizzard fat stores, or fatty acid composition of selected depots of fat (Robel 1972; Dabney and Dimmick 1977; Koerth and Guthery 1987, 1988). Although these techniques have been successfully applied as chronic indices of nutritional status, particularly with respect to energy intake, short-term alterations in diet quality or animal condition are not easily detected by such indices. Techniques for assessing acute changes in protein nutritional status of bobwhite quail are limited.

Altered nutritional states often result in rapid adjustments in physiological homeostasis, resulting in measurable changes in the chemistry or hematology of blood in birds (Hurwitz and Griminger 1961; Leveille et al. 1961; Okumura and Tasaki 1969). Similar approaches have been used to assess alterations in physiology of wild bobwhite quail during the breeding season (Mcrae and Dimmick 1982). The sensitivity of most physiological measures of condition to alterations in diet quality of bobwhite quail are unknown which has limited their use in nutritional assessment; especially, with regards to protein nutrition.

Profiles of free amino acid concentrations in serum and muscle tissue have been used successfully to evaluate the protein nutritional status of laboratory animals (Johnson and Anderson 1982; Gustafson et al. 1986; Tsuda et al. 1989) and humans (Gibson 1990). Concentrations of free amino acids in serum and tissues represent an equilibrium between amino

acid intake in food, rate of use in protein synthesis, and muscle catabolism or amino acid oxidation (Galibois et al., 1987; Larbier et al. 1982). As intake of protein, and to some degree energy, is modified the rates of these physiologic systems which are responsible for maintaining amino acid homeostasis can be expected to change in an animal (Young and Marchini 1990). Clinical approaches are readily available for detecting alterations in amino acid homeostasis in human medicine (Gibson 1990). The ease of obtaining blood or muscle samples in the field and the availability of commercial blood testing laboratories and test kits makes similar clinical approaches to assessing protein nutritional status in wild bobwhite quail attractive.

Our laboratory has been examining alternatives to the traditional morphological indices of condition in quail. Current techniques that assess chronic energy status are of limited use in assessing acute changes in protein nutritional status. In this study we examine the sensitivity of free amino acid pools in serum and muscle tissue of juvenile bobwhite quail to two levels of protein in the diet under controlled experimental conditions. The effects of dietary protein on traditional gravimetric indices of condition are also presented for juvenile and subadult bobwhite quail.

MATERIALS AND METHODS

Animals and Experimental Design

Thirty subadult bobwhite quail (8 weeks old) and 36 juvenile quail (4 weeks old) were weighed, banded, and randomly assigned to one of three experimental diets varying in protein content (8, 15, 33% protein) for a 4- and 3-week trial, respectively (Table 1). Birds were raised under a natural photoperiod in a battery brooder containing vertical decks (4 by 4 by 2 m), located in a well-ventilated housing facility approved for use by the Institutional Laboratory Animal Resources Committee at Oklahoma State University. Temperature in the housing facility was maintained relatively constant using heat lamps and regulating ventilation (Table 1). Water and experimental diets were provided ad libitum throughout the trial.

Experimental protein diets were made isocaloric by varying the concentration of starch (Table 2); crude protein content of each diet was determined by Kjeldahl analysis. Change in body weights was recorded at 2 and 4 weeks for subadult birds and at weekly intervals for juvenile birds. Palatability of diets was assessed by measuring daily food consumption of juvenile birds by offering a known quantity of food and weighing uneaten portions the following day.

Birds were returned to the laboratory after the trials, anesthetized with 5 mg ketamine hydrochloride (Bristol Laboratories, Syracuse, New York), and exsanquinated via the jugular vein. Uncoagulated blood samples were collected in

EDTA (k_3) supplemented tubes and centrifuged at 1000 x g for 10 min at 15 °C. Serum was decanted and stored frozen at -20 °C for amino acid analysis.

Body Condition Analysis

Postmortem examination included weights of the whole body, liver, gizzard, gizzard fat, spleen, adrenal glands, and reproductive organs. Percent change in body weight was determined as 100 X (initial-terminal/initial). Carcasses were dried by lyophilization and ground to a fine powder using a food processor and micro-grinding mill. Body fat was assessed by ether-extraction using a Soxhlet Apparatus (Sawicka-Kapusta 1975) and ash content was determined by combustion in a muffle furnace at 600 °C for 6 hrs. Percent body protein was determined by subtracting percents fat and ash from 100 (Cambell and Leatherland 1980).

Amino Acid Analysis

Fifty mg of breast muscle tissue were homogenized in 300 µl of 0.1N HCL and centrifuged at 1,500 x g for 25 min. Serum and muscle homogenate from each juvenile bird was deproteinized by filtering through a 10,000 molecular weight cut-off ultrafiltration membrane filter (Ultrafree-MC, Millipore, Milford, Mass.) by centrifuging at 1,000 x g for 15 min. An internal standard (25 µl methionine sulfone) was added to 75 µl of the filtered serum and muscle homogenate prior to derivatization. The pre-column derivatization of free amino acids was accomplished with phenylisothiocynate to produce phenylthiocarbamyl amino acids (Pico-Tag

Workstation; Millipore) and re-filtered through a 0.45-μm syringe filter (Acrodisc CRPTFC, Fisher Scientific, Plano, Tex.). Concentrations of 38 individual amino acids were determined in derivatized samples using high pressure liquid chromatography (Water Model 820 system controller and Model 501 pumps; Millipore). The following chromatographic conditions were used: Waters Pico-Tag Silica/C18 (30 cm by 3.9 mm) column; column temperature of 46 °C; flow rate of 1.0 ml/min with back pressure of 5500 psi; system sensitivity of 489 mv/s (recorder) and 0.5 absorbance units full scale (Waters Model 484 UV detector, set at 254 nm); sample size of 10 μl; and 87 min run time. Solvent conditions and gradients used for separation of amino acids were those described by Cohen et al. (1988). Amino acid concentrations were recorded as μmol/dl for serum and nmol/g fresh weight for muscle tissue.

Statistical Analysis

Differences in body condition indices, serum amino acid, and muscle free amino acid concentrations among dietary protein treatments were determined by analysis of variance (PROC GLM; SAS 1988). Two juvenile quail died during the experimental trial and were deleted from all analyses. An unbalanced data set for serum amino acids resulted from our inability to obtain a sufficient volume of serum from 4 juvenile birds fed the 15% protein diet and 1 juvenile bird fed the 8% protein diet. Differences among means ($P < 0.05$) were isolated using the Least Significant

Difference. Relationships among serum and muscle free amino acid levels were explored using Pearson correlation analysis (PROC CORR; SAS 1988).

RESULTS

Subadult Quail

Body Condition Indices-- Initial body weights averaged 140.12 ± 1.51 (SE) g and did not differ ($P = 0.859$) among treatment groups. Two weeks after the trial was initiated the 33 and 15% protein groups were significantly ($P < 0.001$) heavier than the 8% dietary protein group (Fig. 1). Body growth rates, as measured by percent change in body weight during the trial, of 33 and 15% protein-fed chicks were greater ($P < 0.001$) than those fed 8% protein. Percent body weight change averaged -5.40 ± 4.06, 15.17 ± 1.46, and 21.26 ± 2.24% in the 8, 15, and 33% protein groups, respectively. The 33 and 15% protein groups gained an average of 29.04 and 21.29 g respectively but the 8% protein group lost an average of 7.23 g.

Relative liver weight (mg/g body weight) was different ($P < 0.001$) among all dietary protein groups (Fig. 2); there was no difference for absolute liver weight. Absolute and relative weights of the gizzard, spleen, gonad, and adrenal gland did not differ ($P > 0.05$) significantly among dietary treatments. Percent body fat ($P = 0.595$), body protein ($P = 0.432$), and gizzard fat ($P = 0.191$) were not significantly influenced by dietary protein treatment (Fig. 3).

Juvenile Quail

Body Condition Indices-- Initial body weights averaged 51.8 ± 0.8 (SE) g and did not differ ($P = 0.680$) among treatment groups. All 3 isocaloric protein diets were highly palatable and average feed consumption for the 33, 15 and 8% protein treatment groups were 11.0 ± 0.4, 10.0 ± 0.4, and 9.2 ± 0.3 g/bird/day during the trial. Body growth rate differed significantly ($P < 0.001$) among all 3 treatment groups (Fig. 1). Body weight change averaged 38.7 ± 5.3, 82.6 ± 4.9, and 112.2 ± 4.0% in the 8, 15, and 33% protein groups, respectively. The 33, 14, and 8% protein groups gained an average of 58.32, 43.36, and 19.62 g respectively.

Relative liver weight ($P = 0.001$) was significantly heavier for the 8% protein group compared to the 33 and 15% protein groups (Fig. 2); absolute liver weight did not differ among treatments. Absolute and relative adrenal gland, and gonad weights did not differ ($P > 0.050$) significantly among dietary treatments, but absolute weight of the gizzard ($P < 0.001$) was significantly influenced by dietary protein treatment (Fig. 4). Both the 33 and 15% dietary protein groups had weights greater than chicks in the 8% dietary group; differences between the 33 and 15% dietary protein groups were not significant. Absolute and relative spleen weights were significantly ($P = 0.037$ and $P = 0.002$, respectively) influenced by dietary protein (Fig. 4). Weights were higher in the 33 and 15% protein group

than in the 8% protein group; there were no differences between the 33 and 15% protein groups.

Percent body fat ($P < 0.001$) was significantly influenced by dietary protein (Fig. 3). Body fat levels were greater among the 33 and 8% protein groups than the 15% group; differences between 33 and 8% protein groups were not significant. Body protein ($P = 0.002$) also was significantly influenced by dietary protein (Fig 3). Protein comprised a greater percentage of total body dry mass in the 15% protein group than the 8% group. Percent gizzard fat ($P = 0.289$) was not significantly affected by protein intake.

Serum Amino Acids-- Twenty-six amino acids occurred at sufficient concentrations (0.46 μmol/dl) in serum for detection (Table 3). Alanine, glycine, serine, and taurine were the most concentrated amino acids in serum of bobwhite quail. Concentrations of 1-methylhistidine and 3-methylhistidine were low but detected in all individuals.

Total concentrations of neutral (NAA = leucine + isoleucine + tyrosine + phenylalanine + tryptophan) and branch chain amino acids (BCAA = leucine + isoleucine + valine) were significantly ($P < 0.001$ and $P < 0.001$, respectively) lower in 8% than 15 and 33% protein-fed chicks (Fig. 5). Concentrations of sulfur-containing amino acids (SAA = cysteine + methionine) in serum decreased ($P = 0.002$) as protein intake increased. Concentration of aromatic amino acids (AROM = phenylalanine + tyrosine + tryptophan)

tended to be greater ($P = 0.077$) among those fed either a 33 or 15% protein diet compared to 8% protein. Total concentration of essential amino acids (EAA = arginine + threonine + valine + isoleucine + leucine + methionine + phenylalanine + lysine + histidine + tryptophan) decreased greatly ($P = 0.012$) in the 8% protein group in comparison to other diet groups. Concentrations of non-essential amino acids (NEAA = aspartic acid + serine + asparagine + glutamic acid + proline + glycine + alanine + tyrosine) were elevated ($P < 0.001$) among chicks fed 8 or 15% protein compared to 33% protein. As a result, the essential/non-essential (EAA/NEAA) ratio, as calculated by Gustafson et al. (1986), was significantly influenced ($P < 0.001$) by diet; values increased with increased protein in the diet.

With the exception of arginine, histidine, and methionine, concentrations of individual EAA in serum of chicks were greater for the 15 and 33% dietary protein group compared to the 8% group (Table 3). Isoleucine, leucine, lysine, and valine concentrations were more than 2 times greater in the 33% protein-fed group compared to the 8% group. Histidine concentration was significantly higher in the 15% protein group compared to the 8% protein group.

Individual NEAA were less responsive to dietary protein intake in comparison to the EAA (Table 3). However, there was a general tendency for concentrations of NEAA to increase with decreasing protein intake. Alanine, aspartic acid, cysteine, phosphoserine, serine, and taurine

concentrations were greater in the 33% protein group compared to 8% protein group; concentrations of all except aspartic acid were greater in the 15% than 33% protein group and aspartic acid and cysteine were greater in the 8% than 15% protein group.

Ratios of tyrosine/NAA and serine/threonine were significantly lower ($P < 0.001$) in chicks fed a 33% protein diet compared to those on 8% protein (Fig. 5). The ratio of glycine/(leucine + valine) differed significantly ($P < 0.001$) among all diet groups, increasing with decreased protein intake. The branch chain/non-branch chain amino acid (BCAA/NBCAA) ratio differed significantly ($P < 0.001$) among all diet groups as well, decreasing with decreased protein intake. No diet differences for ammonia (overall $\bar{X}$ = 4.84 umol/dl; $P = 0.348$) or urea (overall $\bar{X}$ = 58.64 umol/dl; $P = 0.123$) were detected.

Muscle Free Amino Acids-- Twenty-five amino acids occurred at sufficient concentrations (19.81 nmol/g) in muscle tissue for detection. Alanine, glutamic acid, glycine, serine, and taurine were the most concentrated free amino acids in muscle tissue of bobwhite quail (Table 4). Carnosine, a dipeptide of alanine and histidine, also accounted for a large proportion of the measurable free amino acid pool in muscle. Concentrations of 1-methylhistidine and 3-methylhistidine were not detected in muscle homogenates of experimental subjects.

In general, the total free amino acid pool in muscle tissue tended to be greatest in quail chicks fed a 15% protein diet. Concentrations of NAA ($P = 0.030$) and BCAA ($P = 0.023$) in muscle tissue were significantly lower in 8% protein-fed group compared to those on 15% protein (Fig. 5); concentrations did not differ between the 8 and 33% groups. Concentrations of AROM did not differ ($P = 0.076$) among treatments but the SAA were elevated ($P = 0.001$) in chicks fed the 8% protein diet compared to the other two diets. In contrast, total concentration of EAA in muscle tissue ($P = 0.034$) was depressed among those fed the 8% protein diet; differences in total concentration of NEAA were not statistically significant ($P = 0.078$). The EAA/NEAA ratio was significantly ($P < 0.001$) greater in chicks fed the 15 and 33% protein diets compared to those on the 8% protein diet.

Concentrations of individual EAA in muscle tissue tended to increase in response to moderate (15% protein) protein restriction, but returned to normal (33% protein) or slightly decreased with severe (8% protein) protein restriction (Table 2). Lysine, methionine, and threonine concentrations were greater ($P < 0.05$) in the 15% protein group than the 33% group. Histidine, isoleucine, lysine, threonine, tryptophan, and valine concentrations were greater ($P < 0.05$) in the 15% protein group than the 8% group; tryptophan was the only EAA that was significantly lower among those fed 8% compared to 33% protein in the

diet. Methionine was the only EAA that was significantly greater in the 8% diet group compared to those fed 33% protein.

Among the NEAA, carnosine concentrations were nearly 3-fold greater in the 33% group compared to the 8% protein group ($P < 0.001$; Table 4). In comparison, concentrations of cysteine, glycine, serine, and taurine were significantly greater ($P < 0.002$) among chicks fed 8% than 33% protein. Asparagine, glycine, and serine concentrations were also elevated in the 15% protein group compared to those fed 33% protein. Taurine and glycine concentrations appeared to change in proportion to dietary protein intake.

The ratios of serine/threonine ($P < 0.001$) and glycine/(leucine + valine) ($P < 0.001$) in the muscle were greater in chicks fed 8% protein compared to those receiving either 15 or 33% protein in the diet; the tryptophan/NAA ratio was depressed ($P = 0.045$) in chicks fed either 8 or 15% protein (Fig. 5). The BCAA/NBCAA ratio of chicks fed 8% protein was significantly lower ($P < 0.001$) than those on 15 or 33% protein. There was no significant ($P = 0.140$) difference among treatments for the tyrosine/NAA ratio.

Ammonia concentrations in muscle homogenates were significantly ($P = 0.036$) elevated in the 8 (1705.89 ± 187.93 nmol/g) and 33% (1756.57 ± 187.46 nmol/g) protein groups compared to the 15% (1106.26 ± 121.12 nmol/g) protein group. Urea concentration was significantly ($P = 0.001$) elevated in the 8% (3350.59 ± 308.29 nmol/g) protein group

compared to the 15 (2170.97 ± 178.44 nmol/g) and 33% (2252 ± 121.97 nmol/g) protein groups.

DISCUSSION

Body Condition Indices

Bobwhite quail require a 27% crude protein diet for optimum growth and survival to 6 weeks post-hatch (Nestler et al. 1942, 1944; Baldini et al. 1950, 1953; Tuttle et al. 1953; Andrews et al. 1973; Serafin 1977). The inability of young bobwhite quail to acquire sufficient levels of dietary protein results in depressed growth and development as indicated by reduced body weights of subadult and juvenile quail fed either 15 or 8% protein diets in this study. Body condition indices indicated that juveniles were more sensitive to dietary protein restriction than subadults.

Metabolically active organs such as the liver will often change in size with altered nutritional states, reflecting physiological adaptation to changes in circulating nutrient levels. Liver weight has been shown to respond to different physiological states in ring-necked pheasants (Phasianus colchicus; Anderson 1972) and spruce grouse (Dendragapus canadensis; Pendergast and Boag 1973) and appears to be a useful measure of condition in bobwhite quail chicks. Both subadult and juvenile quail chicks showed increased relative liver weights with increases in dietary protein. Poultry often show similar responses due to increased lipogenisis and fat deposition associated with excessive caloric consumption while trying to meet protein

requirements (Yeh and Leveille 1969; Rosebrough and Steele 1985; Ferket and Sell 1989).

Sizes of the gizzard and spleen were also useful morphometric discriminators for evaluating body condition in juvenile but not subadult quail chicks. The gizzard may serve as a protein reserve source in birds subjected to a dietary protein deficiency through catabolism of muscle protein (Ankney 1977). DuBowy (1985) observed increases in gizzard weight of blue-winged teal (Anas discors) in response to a shift in food quality. Atrophy of major lymphoid organs such as the spleen has been demonstrated in mammals subjected to protein malnutrition during early development (Bell et al. 1976).

Percent body fat levels in juvenile quail also supported our interpretation of liver weight changes that low protein fed chicks consumed an excess of energy relative to their needs as they attempted to meet protein requirements. Auckaland and Morris (1971) and Ferket and Sell (1989) observed similar elevations in body fat of domestic turkey chicks fed protein restricted diets. These findings suggest that some caution must be exercised in using body fat as a measure of overall condition in wild quail (Koerth and Guthery 1987; Dabney and Dimmick 1977).

Free Amino Acids

Arginine, valine, glycine, serine, and taurine in serum and arginine, alanine, glutamic acid, glycine, and serine in muscle were the predominant free amino acids in these two

pools (Table 5), which is consistent with previous studies on poultry (Larbire et al. 1982; Pascaud 1990). In general, concentrations of individual amino acids in serum were not linearly correlated with muscle concentrations. Exceptions included serine ($\underline{r}$ = 0.67), threonine ($\underline{r}$ = 0.51), alanine ($\underline{r}$ = 0.52), tyrosine ($\underline{r}$ = 0.52), valine ($\underline{r}$ = 0.48), and methionine ($\underline{r}$ = 0.67) which were significantly ($\underline{P}$ < 0.01) correlated between serum and muscle pools.

Alterations in amino acid nutrition as a result of consuming diets low in protein has been shown to result in acute changes in rate of amino acid oxidation due to modified enzyme activity and substrate availability, decline in protein synthesis, and altered rates of protein catabolism (Young and Marchini 1990). The net effect of such physiological adjustments is thought to be conservation of important EAA and nitrogen when intake is restricted.

In mammals, consumption of low protein diets usually initiates a greater depression in concentrations of the EAA relative to the NEAA pool in blood serum making the EAA/NEAA ratio a useful index for assessing protein nutritional status in humans (Gibson 1990). The observed decrease in EAA and concomitant increase in NEAA may be partly explained by their inability to synthesize EAA but synthesize NEAA as they are utilized (Whitehead and Dean 1964; Peng and Harper 1970). It has also been postulated that EAA are selectively shunted out of the blood into muscle pool (Lunn et al. 1976). The EAA/NEAA ratio appears to be a useful index for

assesing protein malnutrition in quail chicks; the index increased with increasing protein intake. Shunting of EAA from the serum pool into muscle was indicated by depressed serum concentrations (seven of the 10 EAA measured were significantly depressed in serum of chicks fed the 8% protein diet) relative to muscle (elevated or normal levels for all EAA except tryptophan in 15 and 8% protein groups). Differences in the EAA/NEAA ratio among diet groups were less apparent in muscle tissue. The glycine/(leucine + valine) ratio is frequently used as an abbreviated index of the NEAA (glycine)/EAA (leucine + valine) ratio in human diagnostic medicine (Gibson 1990). The glycine/(leucine + valine) ratio in serum and muscle of quail chicks in response to protein intake was an inverse reflection of the EAA/NEAA ratio.

One of the most useful diagnostic indices of protein nutritional status in humans and laboratory rodents is the concentration of BCAA or the BCAA/NBCAA ratio since BCAA generally accumulate in serum proportionally to dietary concentration (Dreyer 1975; Lunn et al. 1976; Johnson and Anderson 1982; Lunn and Austin 1983; Fashakin and Furst 1987). The BCAA are important mediators of muscle protein synthesis and are actively taken up by muscle (Hutson and Harper 1981). Concentrations of BCAA in both muscle tissue homogenates and serum strongly reflected dietary protein levels in juvenile quail. The depressed concentration of serum and muscle BCAA among low protein-fed quail was

largely due to major reductions in the concentrations of the BCAA isoleucine and valine which is in agreement with observations in protein restricted mammals (Fashakin and Furst 1987). We also observed elevated concentrations of alanine in protein restricted quail chicks which may be related to the catabolism of BCAA. The BCAA are removed by oxidation in the muscle and serve as key nitrogen sources for the transamination of pyruvate to alanine resulting in a net increase in serum alanine concentration (Adibi 1971; Lunn et al. 1976; Antener et al. 1981; Edmonds and Baker 1987).

Concentrations of SAA in both serum and muscle pools were elevated among quail subjected to diets restricted in protein due increases in cysteine and methionine concentrations. Elevations in the SAA, especially cysteine, of quail chicks during protein restriction may have been related to decreased mobilization of cysteine for feather development which occurs rapidly at this age (Munks et al. 1946). Taurine, a non-protein amino acid, is a product of cysteine catabolism in birds and is often elevated in protein malnourished animals (Gustafson et al. 1986; Edmonds and Baker 1987). Muscle and serum taurine concentrations were greatly elevated in protein-restricted quail (200% increase over levels of high protein-fed controls in muscle), which may reflect an increase in cysteine catabolism to taurine rather than pyruvate as a result of the dietary protein deficiency.

Remarkable elevations in concentrations were also evident for alanine, serine, and phosphoserine in serum and glycine and serine in muscle homogenates of protein-restricted quail chicks. For example, glycine levels in muscle of the 8% protein-fed group were on average about 108% greater than high protein-fed controls. Our observations in quail agree favorably with studies by Lunn et al. (1976) with laboratory rats where significant elevations were documented for serine, glycine, and alanine in both plasma and muscle tissues of protein-restricted animals. Elevated serine concentrations may have been related to the elevated levels of glycine which can be converted to serine via serine hydroxymethyl transferase. The large overall increases in concentrations of alanine, glycine, serine, and other NEAA in restricted quail indicates a general adaptation to the high caloric-low protein diets by increasing synthesis of NEAA, possibly as a nitrogen-conserving strategy. Antener et al. (1981) found the serine/threonine ratio to be the most useful index for assessing protein malnutrition in human patients and we observed a substantial elevation in this index among protein malnourished quail chicks.

One of the most prominent alterations in the free amino acid profile of muscle tissue of quail chicks fed protein-restricted rations was the reduction in carnosine concentration. Carnosine, a dipeptide of histidine, occurs at high concentrations in skeletal muscle and acts as a

buffer to lactic acid accumulation when muscle tissue undergoes metabolism, particularly during exercise (DeMasi 1990). Carnosine levels have been shown to vary according to type of muscle fibers and degree of oxidative potential in those fibers. Nutritional induced suppression of carnosine levels in quail could be a response to overall declines in metabolic activity.

Recommended Indices of Condition

Our results support the continued use of traditional morphometric indices such as weights of carcass and selected metabolically active organs to assess nutritional status of developing bobwhite quail. Measures of fat stores are also useful for assessing chronic caloric intake, but do not necessarily provide a useful index of protein intake. It was also apparent that morphological alterations in development are considerably more pronounced, and more useful, in younger aged chicks.

Free amino acid pools of both blood serum and muscle tissue are dynamic and respond to acute alterations in dietary protein. In general, bobwhite quail chicks fed restricted protein responded in a fashion that was remarkably similar to previously documented cases of malnutrition involving humans and laboratory rodents. Several of the diagnostic indices used in clinical nutrition assessment in human medicine appear equally suited for use in quail. Total concentrations of BCAA, especially valine, and ratios of BCAA/NBCAA, EAA/NEAA, glycine/(leucine +

valine), and serine/threonine in blood serum were excellent discriminators among diet groups. Likewise, concentrations of carnosine and taurine and ratios of serine/threonine and glycine/(leucine + valine) were promising discriminators in muscle tissue homogenates.

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Table 1. Experimental design for subadult and juvenile bobwhite quail feeding trials.

	Age Class	
Experimental factors	Subadult	Juvenile
Age	8-weeks old	4-weeks old
Number birds	30	36
Diets (% Crude Protein)	8, 15, 33	8, 15, 33
Mean Temperature	32.3 °C	23.27 °C

Table 2. Ingredient composition of isocaloric 8, 15, 33% protein diets fed to subadult and juvenile bobwhite quail.

	Percent air-dry basis		
Ingredient	8%	15%	33%
Starch	53.31	45.60	30.00
Ground corn	21.67	21.35	20.94
Iso-soy[a]	7.56	16.16	32.66
Animal fat	12.09	11.89	11.63
Dicalcium phosphate[b]	3.32	2.96	2.61
Vitamin mix[c]	0.45	0.44	0.44
Methionine, 99%	0.45	0.44	0.44
Salt	0.45	0.44	0.44
Limestone, 38% Ca	0.45	0.57	0.68
Trace mineral mix[d]	0.15	0.15	0.15

[a] Soybean meal protein, 84.5% crude protein content, Protein technologies International.

[b] 18.5% P, 22% Ca, 0.18% Fl, Pitman-Moore Inc.

[c] Per pound of diet: 1,800,000 IU vitamin A, 500,000 IU vitamin D_3, 6,000 IU vitamin E, 3.6 mg vitamin B_{12}, 1,2000 mg riboflavin, 8,000 mg niacin, 2,000 mg d-pantothenic acid, 90,800 mg choline, 330 mg menadione, 200 mg folic acid, 720 mg pyridoxine, 360 mg thiamine, 20 mg d-biotin; Hoffman-LaRoche Inc.

[d] 15.00% Ca, 10.00% Zn, 12.00% Mn, 7.50% Fe, 1.00% Cu, 0.25% I; J. M. Huber Corp.

Table 3.--Mean (±SE) serum free essential and non-essential amino acid concentrations of juvenile bobwhite quail fed either a 8, 15, or 33% crude protein (CP) diet (μmol/dl).

Amino acid	8% CP (n = 10)	15% CP (n = 8)	33% CP (n = 11)
Essential amino acids			
Arginine[ns]	35.16±2.09	45.68±5.51	42.30±1.70
Histidine	5.65±0.57[b]	9.10±1.09[a]	7.08±0.55[ab]
Isoleucine	6.46±0.96[c]	16.21±3.43[b]	23.48±1.44[a]
Leucine	10.54±1.25[b]	20.82±3.63[a]	22.89±1.25[a]
Lysine	13.63±1.74[b]	39.53±9.52[a]	31.72±4.98[a]
Methionine[ns]	17.54±3.02	16.70±2.29	10.29±0.82
Phenylalanine	10.29±0.66[b]	12.82±1.40[a]	13.25±0.71[a]
Threonine	12.57±0.87[b]	27.34±4.64[a]	22.53±1.53[a]
Tryptophan	1.52±0.14[b]	2.01±0.30[a]	2.37±0.27[a]
Valine	14.51±1.79[c]	32.35±6.01[b]	44.56±2.66[a]
Non-essential amino acids			
Alanine	52.59±5.29[a]	43.37±4.64[a]	29.12±2.20[b]
Asparagine[ns]	16.78±1.44	22.57±1.77	17.95±1.61
Aspartic acid	2.36±0.46[a]	1.23±0.20[b]	1.14±0.17[b]
Carnosine[ns]	1.41±0.23	1.77±0.19	1.95±0.32
Citrulline[ns]	3.43±0.58	3.79±1.00	2.20±0.57
Cysteine	22.97±1.99[a]	17.11±1.88[b]	10.82±0.57[c]
Glutamic acid[ns]	19.84±2.17	21.19±1.88	21.60±1.77
Glycine[ns]	61.41±6.07	54.25±3.18	46.08±2.79
Hydroxyproline[ns]	6.23±1.11	8.65±1.12	7.95±1.24
1-Methylhistidine[ns]	1.99±0.52	2.00±0.23	1.74±0.20
3-Methylhistidine[ns]	3.33±0.52	3.94±0.62	3.52±0.60
Ornithine[ns]	4.19±0.42	7.79±2.01	4.75±0.56
Phosphoserine	6.98±0.92[a]	6.66±1.05[a]	2.66±0.35[b]
Proline[ns]	24.12±1.90	28.77±3.45	21.83±2.30
Serine	69.62±5.65[a]	59.68±4.24[a]	41.44±2.35[b]
Taurine	71.11±6.29[a]	61.61±8.09[a]	42.19±3.93[b]
Tyrosine[ns]	11.35±1.12	12.28±0.77	12.75±0.93

[abc row] Means with the same letter are not different ($P \geq 0.05$).

[ns] No difference among dietary treatments ($P \geq 0.05$).

Table 4.--Mean (±SE) muscle free essential and non-essential amino acid concentrations of juvenile bobwhite quail fed either a 8, 15, or 33% crude protein (CP) diet (nmol/g fresh weight).

Amino acid	8% CP (n = 10)	15% CP (n = 8)	33% CP (n = 11)
Essential amino acids			
Arginine[ns]	679.24±65.91	875.81±60.33	803.25±64.89
Histidine	316.97±41.77[b]	461.20±35.07[a]	399.37±33.88[ab]
Isoleucine	380.49±31.88[b]	570.05±36.44[a]	486.61±35.91[ab]
Leucine[ns]	683.27±70.63	776.64±47.73	623.59±44.65
Lysine	216.02±23.42[b]	311.23±19.77[a]	235.62±20.04[b]
Methionine	419.78±41.84[a]	447.23±24.08[a]	317.52±27.18[b]
Phenylalanine[ns]	344.07±31.19	418.29±28.22	352.10±20.59
Threonine	498.79±42.91[b]	760.64±60.66[a]	594.14±53.74[b]
Tryptophan	98.23±7.81[b]	129.43±7.46[a]	126.63±10.22[a]
Valine	596.67±44.16[b]	834.56±59.59[a]	749.70±58.56[ab]
Non-essential amino acids			
Alanine[ns]	1247.66±88.54	1303.75±82.08	1023.70±81.97
Asparagine	707.83±46.42[b]	911.57±54.41[a]	716.36±66.60[b]
Aspartic acid	366.08±26.56[b]	511.80±36.16[a]	458.92±40.19[ab]
Carnosine	4320.03±752.17[b]	10281.86±810.26[a]	12935.51±1072.16[a]
Citrulline[ns]	48.38±14.26	49.17±8.49	45.37±6.58
Cysteine	393.09±28.74[a]	275.22±27.01[b]	273.95±9.64[b]
Glutamic acid[ns]	1521.65±199.17	1854.97±82.91	1804.48±187.53
Glycine	2133.29±210.31[a]	1534.00±93.27[b]	1024.63±93.73[c]
Hydroxyproline[ns]	64.11±10.65	53.59±8.38	51.42±9.11
Ornithine[ns]	42.71±4.16	39.95±4.44	35.95±11.29
Phosphoserine[ns]	854.38±201.06	901.07±135.30	700.27±106.77
Proline[ns]	484.07±49.04	497.22±37.25	511.65±40.14
Serine	1584.30±92.33[a]	1428.32±71.40[a]	1127.90±75.68[b]
Taurine	2216.31±239.44[a]	1062.92±101.31[b]	741.45±50.09[b]
Tyrosine[ns]	418.87±37.84	509.83±36.90	411.11±34.51

[abc row] Means with the same letter are not different ($P \geq 0.05$).

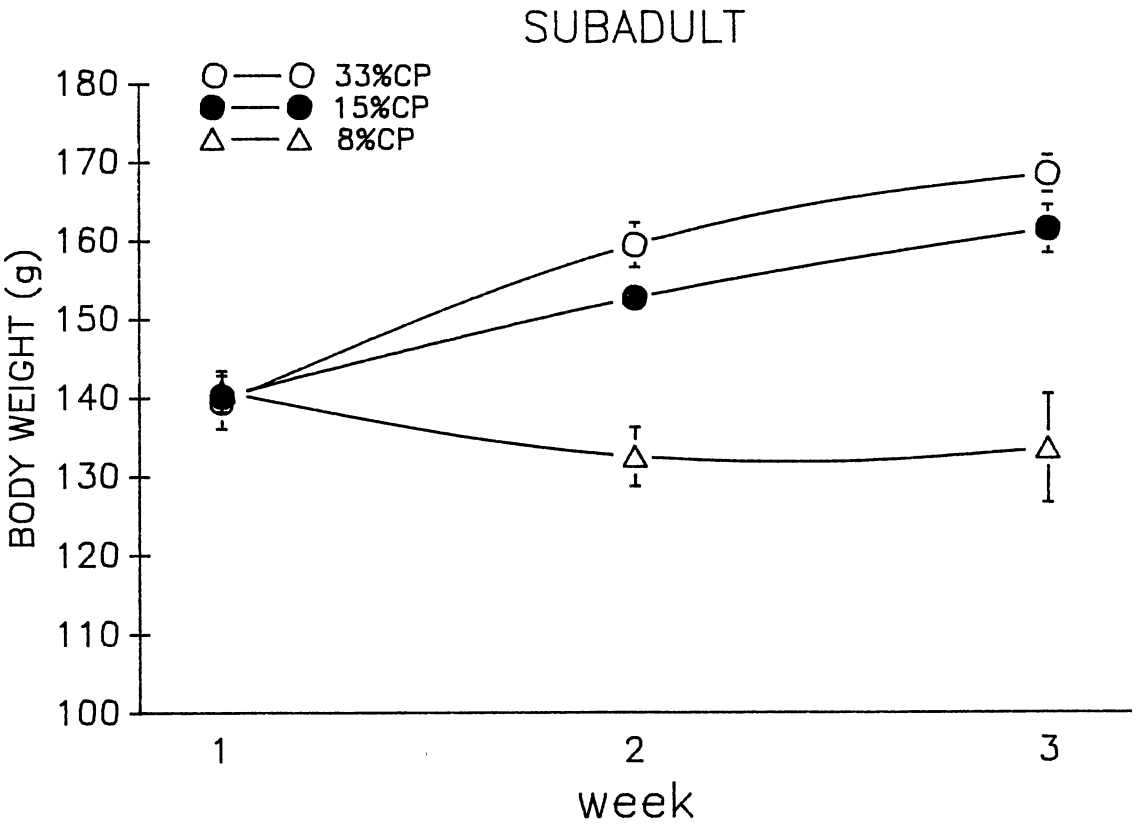
[ns] No difference among dietary treatments ($P \geq 0.05$).

Table 5.--Mean (±SE) serum and muscle free essential and non-essential amino acid concentrations of juvenile bobwhite quail fed either a 8, 15, or 33% crude protein (CP) diet (% total amino acid).

	8% CP		15% CP		33% CP	
Amino acid	Serum	Muscle	Serum	Muscle	Serum	Muscle
Essential amino acids						
Arginine	6.38±0.40	4.09±0.25	7.15±0.52	5.29±0.15	8.75±0.29	7.54±0.17
Histidine	1.15±0.08	1.89±0.17	1.61±0.08	2.78±0.15	1.45±0.06	2.96±0.13
Isoleucine	1.29±0.13	2.31±0.11	2.70±0.34	3.45±0.11	4.86±0.28	3.60±0.07
Leucine	2.11±0.16	4.18±0.34	3.56±0.32	4.70±0.12	4.71±0.17	4.64±0.15
Lysine	2.83±0.33	1.30±0.10	6.46±0.97	1.88±0.05	6.26±0.54	1.74±0.05
Methionine	3.52±0.46	2.56±0.17	3.09±0.38	2.73±0.10	2.10±0.10	2.34±0.10
Phenylalanine	2.10±0.06	2.10±0.12	2.28±0.10	2.53±0.09	2.72±0.08	2.65±0.08
Threonine	2.58±0.14	3.03±0.16	4.84±0.28	4.57±0.22	4.60±0.15	4.33±0.14
Tryptophan	0.31±0.03	0.60±0.03	0.37±0.05	0.79±0.02	0.50±0.06	0.96±0.09
Valine	2.94±0.27	3.64±0.15	5.49±0.58	5.05±0.21	9.18±0.43	5.53±0.10
Total	25.21	25.70	37.55	33.77	45.13	36.29
Non-essential amino acids						
Alanine	10.74±0.82	7.64±0.27	7.7520.62	7.87±0.19	5.97±0.36	7.45±0.17
Asparagine	3.41±0.21	4.34±0.11	4.12±0.25	5.52±0.16	3.65±0.23	5.21±0.17
Aspartic acid	0.47±0.07	2.25±0.10	0.22±0.04	3.09±0.12	0.23±0.06	3.43±0.19
Citrulline	0.71±0.10	0.32±0.10	0.61±0.14	0.30±0.05	0.43±0.11	0.35±0.07
Cysteine	4.72±0.38	2.43±0.14	3.15±0.33	1.68±0.15	2.24±0.11	2.17±0.22
Glutamic acid	4.07±0.38	9.17±0.98	3.91±0.34	11.60±0.80	4.45±0.31	13.28±1.00
Glycine	12.46±0.81	13.61±1.66	9.97±0.56	9.30±0.30	9.45±0.26	7.49±0.25
Hydroxyproline	1.23±0.20	0.43±0.09	1.54±0.12	0.33±0.04	1.65±0.28	0.45±0.11
1-Methylhistidine	0.41±0.03	---	0.37±0.04	---	0.36±0.04	---
3-Methylhistidine	0.71±0.14	---	0.69±0.06	---	0.76±0.15	---
Ornithine	0.84±0.06	0.27±0.03	1.27±0.22	0.24±0.02	0.96±0.06	0.30±0.12
Phosphoserine	1.50±0.22	4.97±0.87	1.20±0.19	5.40±0.67	0.55±0.06	5.08±0.55
Proline	4.90±0.21	2.97±0.23	5.02±0.13	2.99±0.14	4.38±0.20	3.80±0.15
Serine	14.26±0.78	9.88±0.55	11.05±0.99	8.73±0.31	8.55±0.41	8.41±0.19
Taurine	14.61±1.23	13.48±1.05	11.10±1.22	6.53±0.56	8.65±0.65	5.32±0.22
Tyrosine	2.29±0.13	2.54±0.13	2.27±0.16	3.09±0.15	2.60±0.10	3.02±0.08
Total	77.33	74.30	64.24	66.67	54.88	65.76

Carnosine was deleted from table.

Figure 1. Body weights ($\underline{X} \pm \underline{SE}$) of subadult and juvenile bobwhite quail fed formulated isocaloric rations containing either a high, medium, or low protein content.


SUBADULT
33%CP
15%CP
8%CP
BODY WEIGHT (g)
180
170
160
150
140
130
120
110
100
1
2
3
week

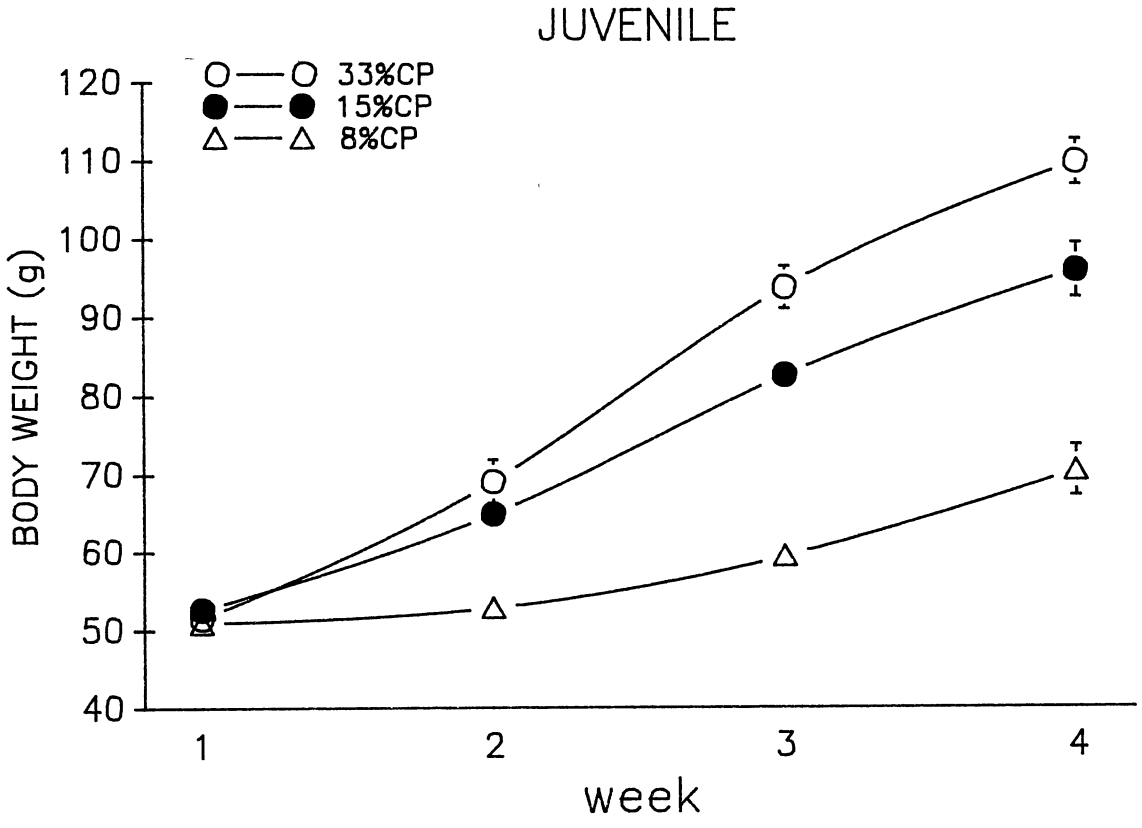
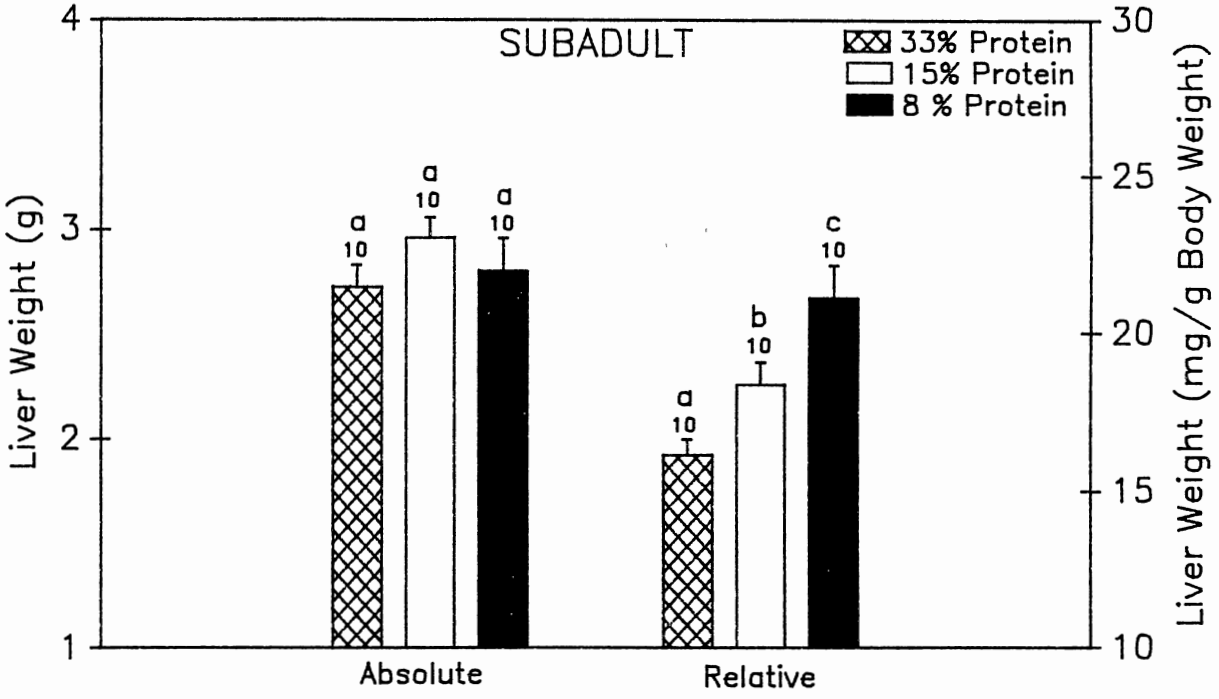

JUVENILE
33%CP
15%CP
8%CP
BODY WEIGHT (g)
120
110
100
90
80
70
60
50
40
1
2
3
4
week

Figure 2. Mean (± SE) absolute (mg) and relative (mg/g body weight) weight of the liver for subadult and juvenile bobwhite quail fed isocaloric diets containing either a 8, 15, or 33% protein over a 3- and 4-week period respectively. Means with different letters are significantly different ($P \leq 0.05$).


SUBADULT
33% Protein
15% Protein
8 % Protein
Liver Weight (g)
Liver Weight (mg/g Body Weight)
a 10
a 10
a 10
a 10
b 10
c 10
Absolute
Relative

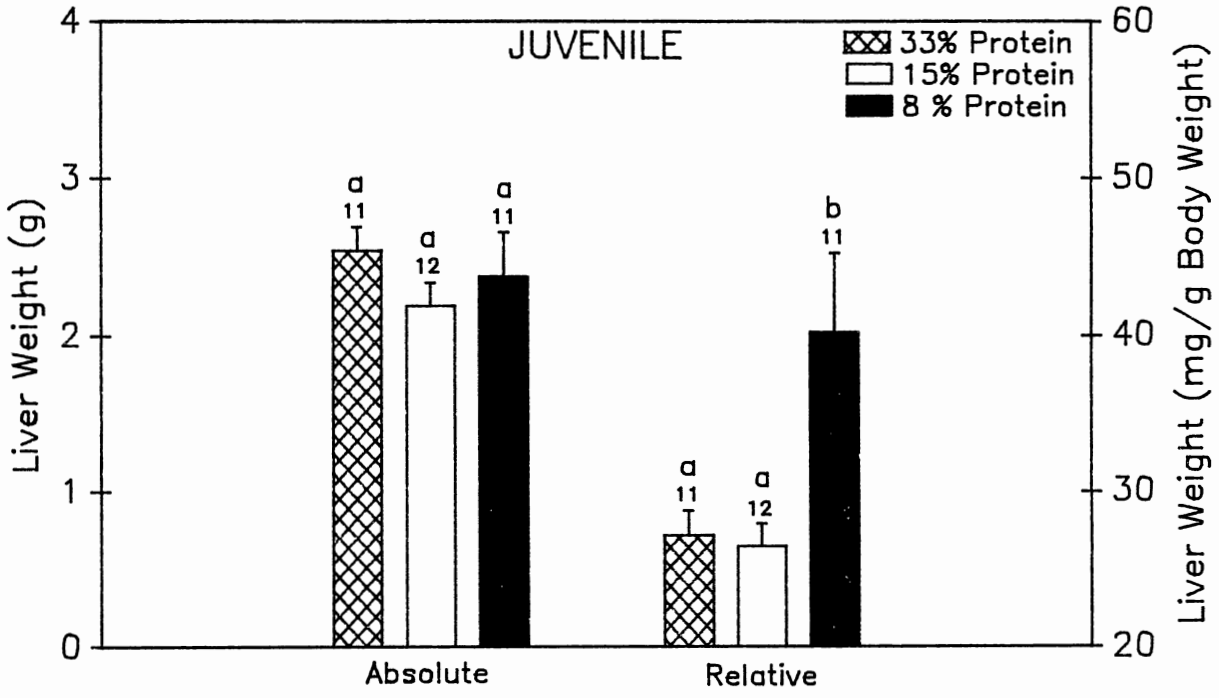
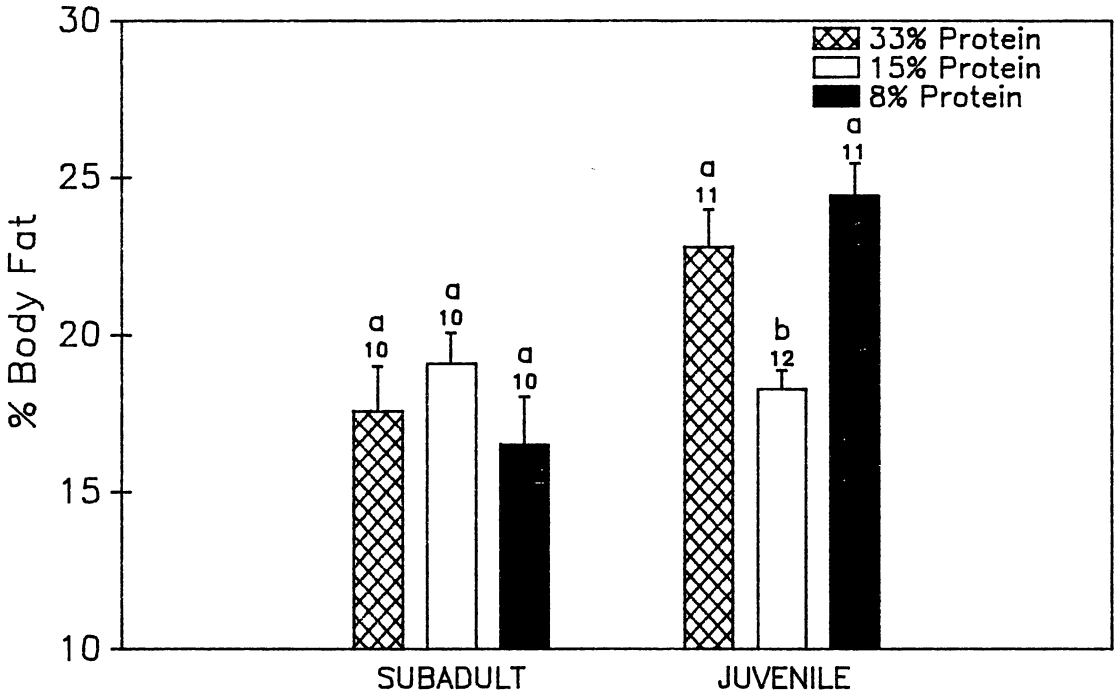

JUVENILE
33% Protein
15% Protein
8 % Protein
Liver Weight (g)
Liver Weight (mg/g Body Weight)
a 11
a 12
a 11
a 11
a 12
b 11
Absolute
Relative

Figure 3. Mean (± SE) percent body fat and body protein for adult, subadult, and juvenile bobwhite quail fed either a high, medium, or low protein diet. Means with different letters are significantly different ($P \leq 0.05$).


30
25
20
15
10
% Body Fat
33% Protein
15% Protein
8% Protein
a
10
a
10
a
10
a
11
b
12
a
11
SUBADULT
JUVENILE

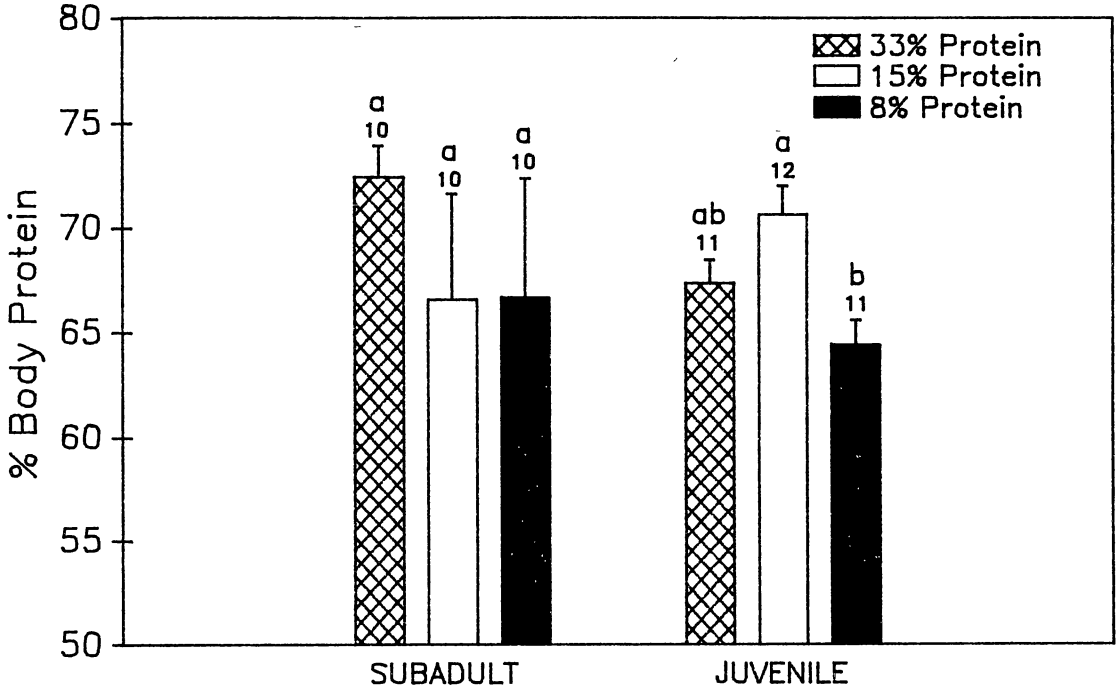
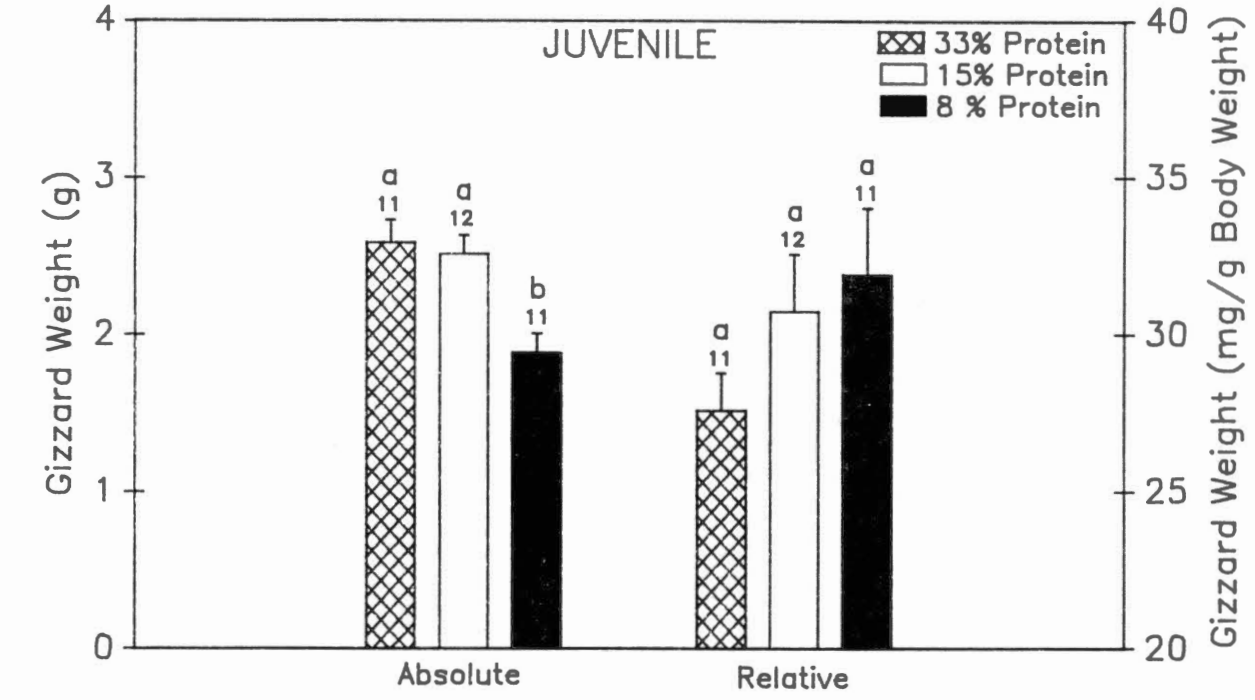

80
75
70
65
60
55
50
% Body Protein
33% Protein
15% Protein
8% Protein
a
10
a
10
a
10
ab
11
a
12
b
11
SUBADULT
JUVENILE

Figure 4. Mean (± SE) absolute (mg) and relative (mg/g body weight) weights of the gizzard and spleen of juvenile bobwhite quail fed isocaloric diets containing either a 8, 15, or 33% protein over a 4-week period. Means with different letters are significantly different ($P \leq 0.05$).


JUVENILE
33% Protein
15% Protein
8 % Protein
Gizzard Weight (g)
Gizzard Weight (mg/g Body Weight)
0
1
2
3
4
20
25
30
35
40
a
11
a
12
b
11
a
11
a
12
a
11
Absolute
Relative

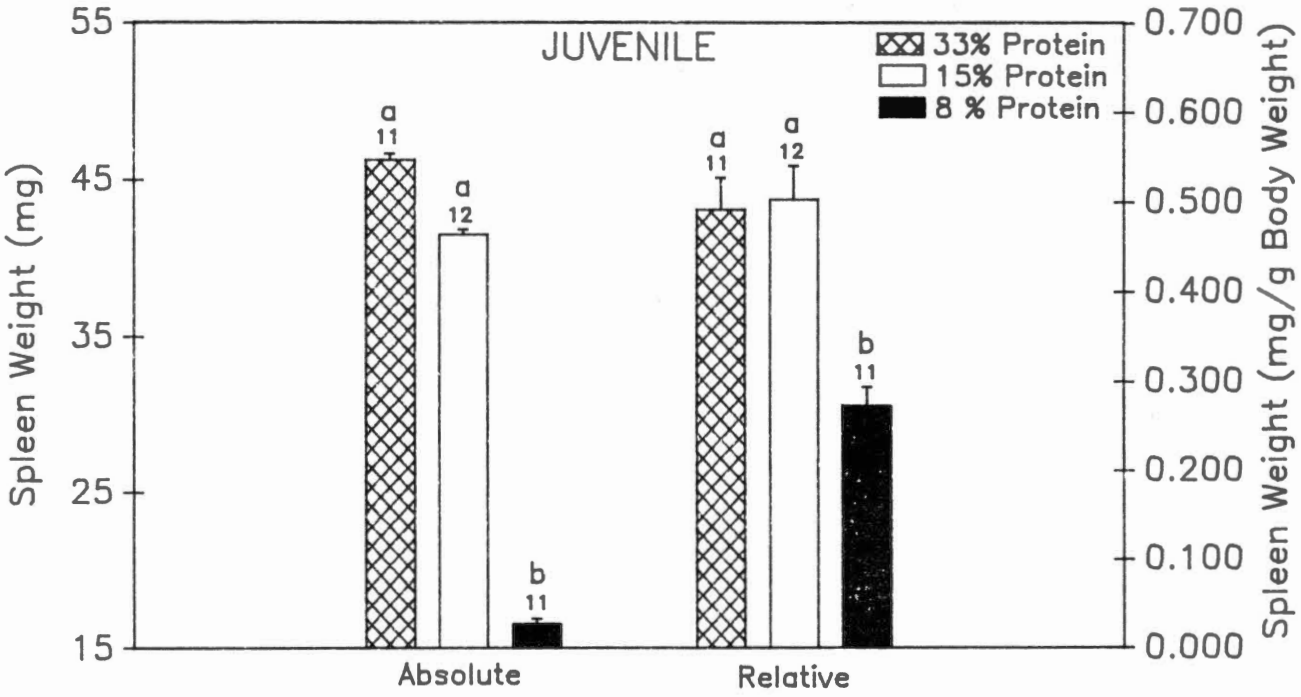
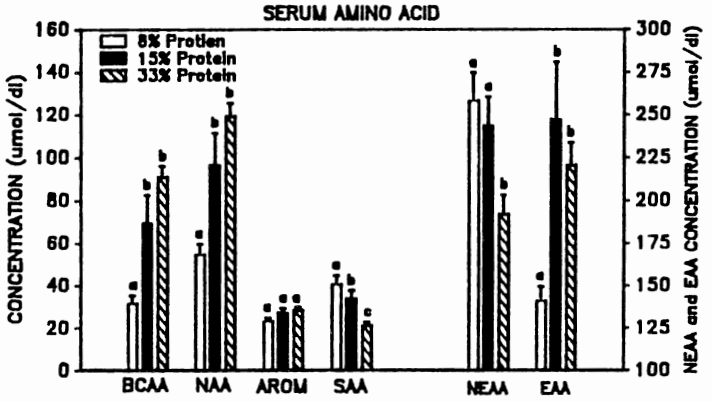
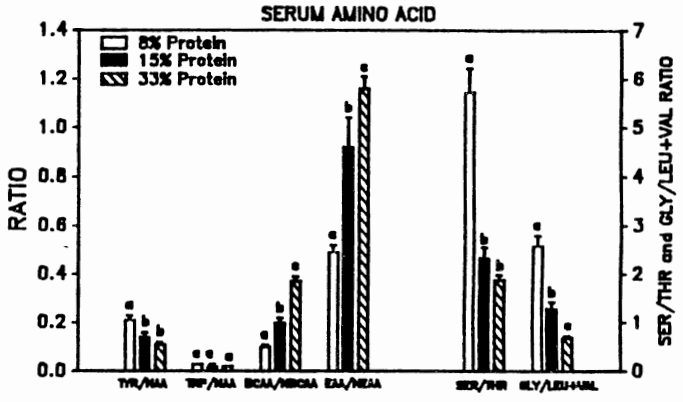
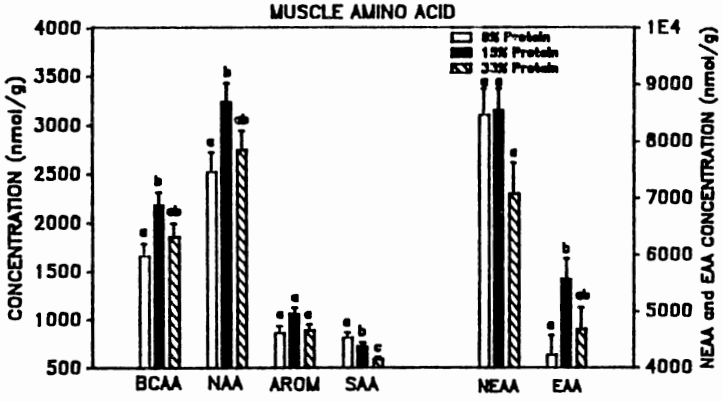
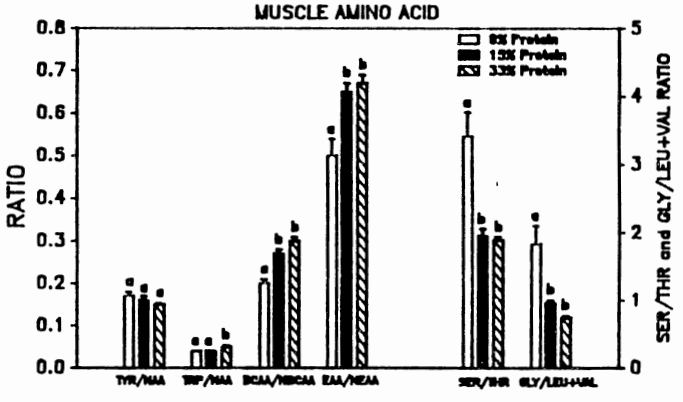

JUVENILE
33% Protein
15% Protein
8 % Protein
Spleen Weight (mg)
Spleen Weight (mg/g Body Weight)
15
25
35
45
55
0.000
0.100
0.200
0.300
0.400
0.500
0.600
0.700
a
11
a
12
b
11
a
11
a
12
b
11
Absolute
Relative

Figure 5. Effect of dietary protein (8, 15, or 33%) on selected free amino acid concentrations and amino acid ratios (BCAA = branch chain amino acids, NAA = neutral amino acids, AROM = aromatic amino acids, SAA = sulfur-containing amino acids, NEAA = non-essential amino acids, EAA = essential amino acids, TYR/NAA = tyrosine/neutral amino acids, TRP/NAA = tryptophan/neutral amino acids, BCAA/NBCAA = branch chain/non-branch chain amino acids, EAA/NEAA = essential/non-essential amino acids, SER/THR = serine/threonine, GLY/LEU+VAL = glycine/(leucine + valine)) in serum and breast muscle of juvenile bobwhite quail. Means with different letters are significantly different ($P \leq 0.05$).


SERUM AMINO ACID
8% Protien
15% Protein
33% Protein
CONCENTRATION (umol/dl)
NEAA and EAA CONCENTRATION (umol/dl)
BCAA
NAA
AROM
SAA
NEAA
EAA


SERUM AMINO ACID
8% Protein
15% Protein
33% Protein
RATIO
SER/THR and GLY/LEU+VAL RATIO


MUSCLE AMINO ACID
CONCENTRATION (nmol/g)
NEAA and EAA CONCENTRATION (nmol/g)
BCAA
NAA
AROM
SAA
NEAA
EAA


MUSCLE AMINO ACID
RATIO
SER/THR and GLY/LEU+VAL RATIO

CHAPTER III

INFLUENCE OF SEASON AND BRUSH MANAGEMENT ON NUTRITIONAL CONDITION AND DIET QUALITY OF BOBWHITE QUAIL

Abstract: We investigated effects of season and brush management on body condition and dietary protein quality of bobwhite quail (Colinus virginianus) in the cross timbers ecosystem. We also evaluated amino acid profiles to assess dietary protein quality and hypothesized that crude protein is an inadequate barometer to assess dietary protein quality for bobwhite quail. Quail were collected seasonally and diet quality was assessed by amino acid analysis using high pressure liquid chromatography. Brush management, including herbicide, herbicide + fire, and mechanical removal, had little long-term effects on body condition or dietary protein quality. However, both body condition and diet quality were significantly altered by season. Essential amino acid (EAA) composition of diets were deficient in meeting growth and reproduction requirements during pre-breeding and breeding period during May and September. In addition, all EAA were deficient in meeting adult maintenance requirement during the winter with the exception of arginine and histidine. Our result indicated that (1)

EAA profiles of crop contents can be used to asses dietary protein quality and (2) dietary EAA may be important in regulating growth, reproduction, and winter survival.

The cross timbers vegetation type is characteristic of central Oklahoma. Because of the dense overstory, forage availability for livestock and wildlife is often limited. Reduction of overstory cover to increase forage for both livestock and wildlife can be achieved by burning and herbicide treatment (Engle et al. 1991) which affects habitat structure, forage availability, and dietary quality for bobwhite quail (Colinus virginianus) (Baumgartner 1945, Ellis et al. 1969, Minser and Byford 1981, Wilson and Crawford 1981, Wiseman and Lewis 1981, Webb and Guthery 1982). These habitat alterations can have both direct and indirect impacts on the nutritional status of quail. Prescribe burning can benefit bobwhite quail by (1) increasing biomass of weed seeds and insects, (2) removing mulch and dead vegetation near the ground surface allowing for increased access to feeding areas, and (3) stimulating new growth with a higher nutritional content (Lang 1954, Dewitt and Derby 1955, Stoddard 1963, Dills 1970, Hurst 1972, Renwald et al. 1978, Umber et al. 1979, Guthery 1986, Koerth et al. 1986, Hansmire et al. 1988, Bogle et al. 1989). Herbicide application also may be beneficial to quail by removing woody vegetation which increases grass and forb production, quail nesting cover, and crude protein content of important quail foods such as common sunflower

(Helianthus annuus) and croton (Croton spp.) (Pletscher and Robel 1979, Umber et al. 1979, Meyer and Bovey 1985, Wood et al. 1986, Guthery et al. 1987).

Populations of bobwhite quail are known to be very unstable and fluctuate widely within and between years. Quail density during fall is highly dependent on nesting success and chick survival (Roseberry and Klimstra 1972, Cantu and Everett 1982). Chick mortality is often high during the first few weeks of development and appears to be related to habitat structure (Wiseman and Lewis 1981, Cantu and Everett 1982, Webb and Guthery 1982). Causes of chick mortality are difficult to assess in the wild, but nutrition is possibly an important consideration (Hurst 1972).

Considerable efforts have been devoted to documenting crude protein in foods (Nestler et al. 1945, Newlon et al. 1964) and diets (crop contents, Wood et al. 1986) of bobwhite quail. Most studies on protein requirements of bobwhite quail have been conducted without regard to biological quality of proteins. A high biological value results when dietary protein meets daily intake requirements for essential amino acids (EAA). Availability and composition of EAA in forage and diets of wild bobwhite quail are completely unknown. We hypothesized that amino acid composition of the diet may be deficient in meeting dietary requirements for growth, reproduction, and winter maintenance.

Our primary objective was to determine if habitat modification using prescribed burning or herbicide influences the nutritional ecology of bobwhite quail as measured by gross body condition and diet quality. We also evaluated amino acid profiles to assess dietary protein quality.

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STUDY AREA

The Cross Timbers Experimental Range (CTER) is located in Payne County, Oklahoma (36°2' to 36°4'N, 97°9' to 97°11'W) in the cross timbers land resource area (Garrison et al. 1977). Vegetation on CTER is dominated by post oak (Quercus stellata) and black-jack oak (Quercus marilandica) in the overstory with interspersed tallgrass prairie (Ewing et al. 1984). For each of the 4 habitat types, bobwhite quail were collected from 4 different 32-ha sample plots. Three habitats were altered by removal of woody vegetation in the overstory, and one habitat was an unmodified control. The three altered habitats were: (1) herbicide only, (2)

herbicide followed by annual burning, and (3) mechanical brush removal. Herbicides included tebuthiuron or triclopyr applied aerially at 2.2 kg/ha. Detailed descriptions of the study area have been previously published (Engle et al. 1991, Lochmiller et al. 1991, Stritzke et al. 1991).

METHODS

Sampling Intensity

Bobwhite quail were collected seasonally (May 1989 and 1990, Sept 1990 and 1991, Feb 1989 and 1990). Due to low collection numbers, birds were pooled between years within each season. A total of 67, 62, and 37 birds was harvested during May, September, and February, respectively.

Body Condition Analysis

Postmortem examination included weights of the whole body, liver, gizzard, gizzard fat, spleen, adrenal glands, and reproductive organs. The small intestine was flushed and placed in the carcass. The carcass and the contents of the crop, gizzard, ceca, and large intestine were bagged individually, dried by lyophilization, and ground to a fine powder using a food processor and a micro-grinding mill. Body fat was assessed by ether-extraction using a Soxhlet Apparatus (Sawicka-Kapusta 1975) and ash content was determined by combustion in a muffle furnace at 600 C for 6 hrs. Percent body protein was determined by subtracting percents fat and ash from 100 (Cambell and Leatherland 1980).

Diet Quality Analysis

Nutritional quality of quail diets were evaluated indirectly by crop content nutrient analysis. Subsampling within treatments was performed when crop samples were <0.2 g. Due to empty crop contents in some birds, 45, 33, and 19 crops were analyzed from the May 1989 and 1990, September 1990 and 1991, and February 1989 and 1990 collections, respectively. Percent fat of crop and gizzard contents was assessed by ether-extraction using a Soxhlet Apparatus (Sawicka-Kapusta 1975). Total nitrogen pool of digesta in the crop, cecum, and gizzard was determined by micro-Kjeldahl analysis using a 0.1 g sample (Williams 1984).

Fat-extracted crop contents amounting to approximately 40 mg of protein were weighed into 25 X 150 mm glass tubes with teflon caps and hydrolyzed in 15 ml 6N HCL at 110 C for 24 hr. One ml of the hydrolyzed sample was filtered through a 0.45-μm syringe filter (Acrodisc CRPTF, Fisher Scientific, Plano, Tex.). An internal standard (25 μl methionine sulfone) was added to 75 μl of the filtered hydrolosate before derivatization. Pre-column derivatization of amino acids was accomplished using phenylisothiocynate to produce phenylthiocarbamyl amino acids (Pico-Tag Workstation, Millipore Corporation, Milford, Mass.) and re-filtered through a 0.45-μm syringe filter. Concentrations of 17 individual amino acids were determined in derivatized crop samples using high pressure liquid chromatography (HPLC,

Waters Model 820 system controller and Model 501 pumps). Tryptophan was destroyed by acid hydrolysis and therefore not measured. Chromatographic conditions were the following: Waters Pico-Tag Silica/C18 (15 cm X 3.9 mm) column; column temperature of 38 C; flow rate of 1.0 ml/min with pumps back pressure of 5500 PSI; system sensitivity of 489 mv/sec (recorder) and 0.5 absorbance units full scale (Waters Model 484 UV detector, set at 254 nm); sample size 4 μl; and run time 28 min. Solvents used were Eluent A and Eluent B (catalog no. 88108 and 88112, respectively), Millipore Corporation (Milford, Mass.). Solvent conditions and gradients used for separation of amino acids were those described by Cohen et al. (1988). A casein reference protein (from bovine milk, no. C-0376, Sigma Chem. Co.) of known amino acid composition was hydrolyzed and analyzed with crop samples for comparison. Amino acid concentrations were recorded as a relative proportion of the total amino acid pool and on a dry weight basis.

Statistical Analysis

Differences in body condition indices and dietary quality (including amino acid composition) among the 3 collection seasons, 4 habitat types, and between unmodified and modified habitats were tested by analysis of variance with habitat type and season as the factor terms (PROC GLM, SAS 1988). Interactions of habitat type, season, and sex were examined. We used Least Significant Difference test to isolate differences ($P \leq 0.05$) among means in the presence

of a significant F-test. Relationships among body condition indices and crop content amino acid levels were explored using Pearson correlation analysis (PROC CORR; SAS 1988).

RESULTS

Body Condition Indices

Season x treatment interactions were significant ($P \leq 0.05$) for only absolute and relative gizzard and gizzard fat weights. Season x sex interactions for weights of the whole body, liver, gizzard fat, and percent body fat, body protein, and gizzard fat content were significant. The only significant season x treatment x sex interactions were for percent body protein and gizzard fat content.

Season.--Sixteen of the 20 body condition indices were affected by season (Table 1). Whole body weight was heavier for quail collected in May and February compared to September. Absolute liver and reproductive organ weights were significantly greater in May birds compared to September and February birds; relative liver weight was significantly lower in February birds compared to May and September birds. In addition, absolute and relative liver weight were significantly ($P < 0.001$) greater for females (3.36 ± 0.61 SE and 18.44 ± 0.71 g, respectively) than males (2.59 ± 0.05 and 15.65 ± 0.30 g, respectively). Absolute gizzard weight was greater in February birds compared to May or September birds; however, relative gizzard weight was significantly greater in September birds followed by February and May birds, respectively. Relative and absolute

gizzard fat weight and percent body fat were elevated in birds collected in February compared to May and September while percent body protein was lower in February birds. Protein comprised a lower percentage of total body dry mass in the February birds compared to the May and September birds. Percent protein of the gizzard and cecum contents were greater in birds collected in May and September compared to February, but percent fat of the gizzard contents was higher in birds collected in February followed by September and May, respectively. A positive correlation ($P < 0.001$) existed between body weight and body fat ($r = 0.302$).

Habitat Modification.--Only 8 of the 20 body condition indices differed among habitat types (Table 2). Weights of the whole body, absolute and relative liver, relative gizzard fat, and percent fat of the gizzard contents were greater in birds collected from the control treatment compared to the mechanical treatment. Birds collected from the unmodified habitat had significantly ($P \leq 0.05$) greater body weight (180.35 ± 2.68 and 167 ± 3.05 g), absolute liver weight (3.01 ± 0.12 and 2.78 ± 0.09 g), gizzard protein content (22.30 ± 0.72 and 20.96 ± 0.70 %), and percent gizzard fat content (10.57 ± 0.62 and 9.76 ± 0.62 %) compared to modified habitats.

Dietary Protein and Amino Acid Analysis

Season x treatment interactions (on a percent total amino acid basis) were significant ($P \leq 0.05$) for only

glycine, arginine, proline, tyrosine, leucine, phenylalanine, and lysine. No interactions occurred when amino acids were expressed on a percent dry weight basis. Crude protein, fat, and amino acid levels of crop contents did not differ significantly ($P \geq 0.05$) between sexes and there were no significant season x sex or season x treatment x sex interactions.

Season.--Percent crop protein was significantly greater in May birds followed by September and February birds, respectively (Table 3). However, percent crop fat was significantly greater in February birds followed by May and September birds, respectively.

With the exception of glucine, proline, and tyrosine, concentrations of individual amino acids of crop contents (% dry weight basis) were significantly influenced by season (Table 3). All of the EAA concentrations, with the exception of arginine, were significantly greater in crop contents of May birds compared to February birds. Histidine, isoleucine, and methionine concentrations of September crops did not differ significantly between May or February. Of the non-essential amino acids (NEAA), alanine, aspartic acid, cystine, glycine, and serine concentrations were greater in crop contents of May birds compared to February birds. Concentrations of individual EAA, when expressed as a percentage of the total amino acid pool, were effected less by season (Table 4). Lysine, threonine, and valine concentrations were significantly greater in crop

contents of May birds compared to September and February birds; differences between September and February were not significant. However, arginine concentration was greatest in February crops followed by May and September crops respectively, but leucine concentration was greatest in September crops followed by May and February. Aspartic acid and glucine concentrations were higher in February crops compared to May crops. Alanine concentration was higher in September crops compared to May and February while glycine concentration was higher in May crops compared to September and February crops.

Habitat Modification.--Percent crop protein was not significantly different among brush treatments (Table 5). Percent fat of crop contents was significantly greater in birds collected from the herbicide + fire treatment compared to the other brush treatments and lowest in crop contents of birds collected from the mechanical treatment; differences between the herbicide and control were not significant.

Crops from birds collected from the unmodified habitat had significantly greater concentrations (expressed on a dry weight basis) of aspartic acid (1.27 ± 0.08 and 1.16 ± 0.07), glutamic acid (2.28 ± 0.16 and 2.01 ± 0.10), arginine (0.96 ± 0.06 and 0.84 ± 0.06), and lysine (0.73 ± 0.07 and 0.67 ± 0.05) compared to modified habitats. However, only 2 of the 20 amino acid concentrations of crop contents expressed on a percent dry weight basis were significantly altered among individual brush removal treatments (Table 5).

Lysine and aspartic acid concentrations were greater in crops from birds collected from the herbicide treatment compared to the herbicide + fire. Histidine, leucine, and phenylalanine concentrations, when expressed as a percent total amino acid pool, were greater in crop contents of birds collected from the mechanical treatment compared to the control (Table 6). Lysine and glucine concentrations of crop contents were lower in birds collected from the mechanical treatment compared to the control.

DISCUSSION

Body Condition Indices

Morphological and physiological consequences of moderate to low dietary protein deficiencies in upland game birds are not completely known, but reduced growth and development, body protein and fat, and gizzard fat have been documented under controlled conditions (Neave and Wright 1968, Anderson 1972, Robel 1972, Pulliainen 1976, Dabney and Dimmick 1977, Wood et al. 1986, Koerth and Guthery 1987, 1988).

Differences in body weight are due to the different age classes of birds collected during the 3 collection periods. Birds collected in May and February were all adults, but 69 and 13% of the birds collected during September were subadult and juveniles, respectively, which resulted in a significant reduction in whole body weight during September.

Body condition of birds has been assessed through quantifiable measures such as the size of protein and fat

reserves (Sears 1988, DuBowy 1985, Kirkpatrick 1980). In addition, studies have found a significant positive relationship between body weight and percent body fat (Dabney and Dimmick 1977, Koerth and Guthery 1988) as we observed in our study. Previous studies have observed decreases in percent body fat and gizzard fat weight from winter to spring (Koerth and Guthery 1987). Similarly, we found >50% reduction in percent body fat and relative gizzard fat weight from February to May. Changes in percent body fat and gizzard fat weight are due to changes in dietary fat intake levels. During February quail rely on seeds to meet their dietary requirements which are typically high in fat and low in protein; however, in May and September quail consume large quantities of insects which are low in fat and high in protein (Hurst 1972). Body protein also was elevated in birds collected in May and September due to change in relative body fat stores.

Absolute gizzard weight was higher for birds collected in February compared to May and September due to seasonal changes in feeding strategies. The depressed gizzard weight during the pre-breeding and breeding period (May and Sept) may be due to an increase in the availability of soft foods, such as insects, earthworms, and water-softened seeds (Anderson 1972, DuBowy 1985), which resulted in atrophy of the gizzard. Relative organ weights of the digestive system are greatest in birds at the time of the rapid body growth (14-19 weeks) due to increased food consumption and

digestive activity (Kirkpatrick 1944), which may explain why relative gizzard weight was elevated in quail collected in September.

Liver will often change in size with altered nutritional states, reflecting physiological adaptation to changes in circulating nutrient levels (Anderson 1972, Pendergast and Boag 1973). Increased relative and absolute liver weight of quail during May compared to February was probably a response to increased dietary protein and estrogen secretion in hens, which is at maximum levels at this time (Anderson 1972). Estrogen promotes accumulation of fat and protein in the liver (Common et al. 1948, Ljunggren 1968). In addition, reproductive organ weights were elevated during May which corresponds to the pre-breeding and breeding period of bobwhite quail in Oklahoma (Sutton 1967)

Less than half of the body condition indices were affected by vegetation management. Because season x treatment statistical interactions were significant ($P \leq 0.05$) for only gizzard and gizzard fat weight, treatment effects likely reflected normal seasonal-related alterations in physiology and diet and not effects of brush treatments.

Dietary Protein and Amino Acid Analysis

Bobwhite quail populations in the Midwest exhibit erratic annual fluctuation in density (Rosebery and Klimstra 1984). Bobwhite quail require 23-27% crude protein diet for optimum growth and reproduction (Nestler et al. 1942,

Baldini et al. 1953, Tuttle et al. 1953, Andrews et al. 1973) and 11-12% crude protein for maintenance in winter (Nestler et al. 1944). Protein shortages may regulate chick survival (Hurst 1972), and winter is considered a major period of nutritional stress for adult birds (Robel 1965, Robel and Fretwell 1970). The long-term consequences of protein malnutrition during winter and early spring on reproduction are unknown for quail, but studies of red grouse (Lagopus seotieus) suggest a strong relationship exists (Moss 1973).

Dietary proteins are composed of 20 individual amino acids. Birds do not synthesize adequate quantities of about 10 amino acids (EAA), which must be consumed in the diet to meet daily requirements (Munks et al. 1946, Robbins 1983). Dietary deficiencies of an EAA may lead to reduced growth, reproductive performance, immunocompetence, or survival (Baldini et al. 1953, Okumura and Mori 1979, Robel 1979, Allen and Young 1980, Begearmi et al. 1982, Harms and Buresh 1987, Tsiagbe et al. 1987, Klasing and Barns 1988).

Crop protein was highest in May followed by September and February due to seasonal dietary changes. Upland game birds consume greater proportions of insects during the breeding season to meet dietary protein and amino acid requirements (Hurst and Poe 1985). Most of the amino acid concentrations of the crop contents, when expressed on a percent dry weight basis, were significantly altered by season due to changes in dietary protein intake. The

decrease in crude protein in the diet during February resulted in significant reductions in most of the EAA and NEAA compared to May. Amino acid concentrations of crop contents, when expressed relative to total amino acid pool, were less affected by seasonal changes. Differences in percent total amino acids indicates that the quality of proteins as measured by the amino acid composition varied among dietary food protein.

Dietary requirements for quail have been published (National Research Council 1984) and are routinely reported as a percent dry weight of diet (Table 7). We compared the concentrations of each EAA in the crop contents for each collection season (Table 3) to dietary requirements for growth, reproduction, and winter maintenance. All EAA, with the exception of histidine were deficient during May and September for growth and reproduction. However, only methionine + cystine and phenylalanine were deficient in meeting dietary requirements for adult maintenance during pre-breeding and breeding (May and Sept). All EAA of February crop contents were deficient in meeting adult maintenance requirements with the exception of arginine and histidine. Tryptophan, an EAA comprising about <1% of the total dry weight of seeds (Harrold and Nalewaja 1977), was not measured in crops because it is destroyed by acid hydrolysis. Some loss (about 15%) of methionine and cystine undoubtedly occurred as well due to varying degrees of destruction in acid hydrolysis (Spindler et al. 1984, Elkin

and Griffith 1985). Sulfur-containing EAA methionine and cystine were most limited in crops (even considering analytical inaccuracies). Dietary crude protein requirements were met for juvenile quail during May and September and adult birds during February. However, amino acid composition of the diet was highly deficient in meeting growth, reproduction, and winter maintenance requirements of bobwhite quail.

Because season x treatment interactions were not apparent, habitat type did not appear to account for the observed variation of amino acid composition of crop contents between seasons because crop protein was not altered among brush treatments. Quality of forbs and grasses increase following brush control, but improvement of crude protein content of forages were limited only to the growing season following treatment (Masters and Scifres 1984, Biondini et al. 1986).

Studies have found that high herbicide application rates (>0.8 kg/ha of tebuthiuron) result in a decline of forb biomass and diversity (Scifres and Mutz 1978, Pletscher and Robel 1979, Umber et al. 1979, Baker et al. 1980, Jones 1982, Doerr and Guthery 1983, Masters and Scrifes 1984, Meyer and Bovey 1985). However, Doerr and Guthery (1983) found no significant difference in abundance and diversity of insects on tebuthiuron treated areas. Studies examining whether amino acid composition of specific plant proteins are influenced by soil fertility or other habitat

alterations have been contradictory. Robinson (1975) provided strong evidence that environment can alter relative proportions of amino acids in various kinds of protein in cultivated sunflower seeds. In contrast, several other studies found no significant alteration by nitrogen fertilization of amino acid profiles of cultivated crops (Eppendorfer 1977, Meredith and Gaskins 1984, Meredith et al. 1984). This suggests that habitat alterations may not significantly effect amino acid composition of dietary food proteins as observed in our crop samples.

MANAGEMENT IMPLICATIONS

Our data suggest that EAA analysis of crop contents are useful in assessing dietary protein quality of bobwhite quail. Dietary protein quality, based on crop EAA composition, was most limiting for adult quail during the winter which paralled high body fat stores which could explain low population densities during late winter in some years. In addition, dietary EAA were deficient in meeting requirements for growth and reproduction during the pre-breeding and breeding season, which suggested that EAA of dietary food protein regulate, to some degree, reproductive performance in wild quail. These data strongly support management practices that would increase insect densities and forage diversity in order for quail to meet dietary amino acid requirements.

Previous studies indicate that prescribed burning and herbicide application may increase quail densities by

increasing feeding areas, nesting cover, and forb biomass; however, our data suggest that these brush management techniques do not alter the nutritional condition or dietary protein quality of quail in the cross timbers ecosystem. Based on our results and coupled with previous studies on improving quail densities, vegetation management could be implemented to increase quail densities without significant long-term alterations in body condition or dietary protein quality.

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Table 1. Seasonal effects on selected body condition indices of bobwhite quail ($\bar{X}$ ± SE).

Indices	Season			
	May	September	February	(P > F)
Whole body, g	181.24±1.96[a]	157.14±4.20[b]	185.27±3.29[a]	0.0001
Liver, g	3.25±0.12[a]	2.57±0.09[b]	2.75±0.17[b]	0.0001
mg/g body weight	17.63±0.51[a]	16.76±0.52[a]	15.00±0.86[b]	0.0003
Gizzard, g	7.59±0.14[b]	7.64±0.22[b]	8.35±0.26[a]	0.0079
mg/g body weight	41.96±0.70[c]	49.57±1.27[a]	45.24±1.34[b]	0.0001
Spleen, mg	4.96±0.98	4.91±0.45	2.19±0.49	0.1710
mg/g body weight	0.28±0.06	0.31±0.03	0.11±0.02	0.0807
Adrenal gland, mg	1.26±0.06	1.23±0.15	1.15±0.10	0.5794
mg/g body weight	0.07±0.00	0.08±0.01	0.06±0.00	0.1839
Gonad/Ovary, mg	149.11±11.46[a]	23.36±15.19[b]	7.38±4.78[b]	0.0001
mg/g body weight	8.22±0.60[a]	1.20±0.75[b]	0.40±0.26[b]	0.0001
Gizzard fat, mg	7.99±1.29[b]	4.78±0.54[b]	25.36±3.35[a]	0.0001
mg/g body weight	0.43±0.07[b]	0.29±0.03[b]	1.37±0.18[a]	0.0001
Body fat, %	7.47±0.36[b]	7.76±0.41[b]	19.79±1.25[a]	0.0001
Body protein, %	83.26±0.36[a]	81.81±0.44[a]	71.96±1.19[b]	0.0001
Ash, %	9.35±0.18[b]	10.44±0.24[a]	8.25±0.20[c]	0.0001
Water, %	64.94±0.36[b]	67.70±0.31[a]	60.43±0.52[c]	0.0001
Gizzard protein, %	22.45±0.78[a]	22.68±0.85[a]	18.07±83.60[b]	0.0007
Gizzard fat, %	6.19±0.35[c]	10.56±0.54[b]	16.50±0.87[a]	0.0001
Cecum protein, %	42.70±0.93[a]	44.18±0.87[a]	34.97±1.15[b]	0.0001

[abc] row Means with same letter are not different ($P \geq 0.05$).

Table 2. Treatment effects on selected body condition indices of bobwhite quail ($\underline{X} \pm \underline{SE}$).

Indices	Treatment				
	Herbicide	Herbicide + Fire	Mechanical	Control	($\underline{P} > F$)
Whole body, g	171.96±4.33^{ab}	165.87±4.87^{b}	166.13±5.90^{b}	180.35±2.69^{a}	0.0403
Liver, g	2.95±0.15^{ab}	2.84±0.16^{ab}	2.54±0.14^{b}	3.01±0.12^{a}	0.0042
mg/g body weight	17.03±0.54^{ab}	17.32±0.74^{ab}	15.48±0.71^{b}	16.78±0.58^{a}	0.0183
Gizzard, g	7.61±0.27	7.67±0.27	7.15±0.36	8.12±0.14	0.2709
mg/g body weight	44.85±2.05	47.32±1.58	43.08±1.32	45.63±0.98	0.5774
Spleen, mg	5.49±2.31	3.37±0.35	3.90±0.98	4.70±0.62	0.5941
mg/g body weight	0.32±0.13	0.21±0.02	0.24±0.06	0.26±0.04	0.7461
Adrenal gland, mg	1.08±0.10	1.13±0.06	1.25±0.10	1.31±0.13	0.5666
mg/g body weight	0.06±0.01	0.07±0.00	0.08±0.01	0.07±0.01	0.5991
Gonad/Ovary, mg	116.72±28.05	42.85±10.44	84.24±17.93	65.68±15.69	0.5596
mg/g body weight	6.50±1.47	2.38±0.56	4.72±1.00	3.50±0.80	0.5454
Gizzard fat, mg	08.00±2.03	10.64±3.16	6.21±1.44	13.24±1.53	0.1056
mg/g body weight	0.45±0.11^{ab}	0.60±0.17^{ab}	0.34±0.07^{b}	0.71±0.08^{a}	0.0371
Body fat, %	7.49±0.74	9.43±1.01	9.27±1.03	12.06±0.88	0.9199
Body protein, %	82.89±0.66	80.93±0.93	80.71±0.98	78.82±0.82	0.8723
Ash, %	9.62±0.46	9.65±0.31	10.06±0.25	9.20±1.20	0.5263
Water, %	64.80±0.70^{bc}	65.45±0.61^{b}	66.92±0.52^{a}	64.01±0.47^{c}	0.0474
Gizzard protein, %	24.51±1.79^{a}	19.77±0.86^{c}	20.15±1.18^{bc}	22.30±0.72^{ab}	0.0070
Gizzard fat, %	7.56±0.66^{b}	11.85±1.07^{a}	8.06±0.86^{b}	10.57±0.63^{a}	0.0098
Cecum protein, %	45.52±1.30^{a}	42.13±1.16^{ab}	38.73±1.14^{b}	41.00±1.07^{b}	0.0575

$^{abc\ row}$ Means with same letter are not different ($\underline{P} \geq 0.05$).

Table 3. Seasonal effects on amino acid concentrations of bobwhite crop contents. Amino acids expressed on a percent dry weight basis ($\underline{X}$ ± $\underline{SE}$).

Amino acid	Season			
	May (n = 45)	September (n = 33)	February (n = 19)	($\underline{P}$ > F)
Crop protein, %	29.26±1.17[a]	26.52±1.18[b]	19.60±1.41[c]	0.0017
Crop Fat, %	15.32±1.22[b]	10.00±0.73[c]	21.83±2.55[a]	0.0001
Essential amino acids				
Arginine	0.985±0.06[a]	0.802±0.08[b]	0.796±0.06[ab]	0.0427
Histidine	0.591±0.05[a]	0.451±0.04[ab]	0.328±0.04[b]	0.0365
Isoleucine	0.705±0.05[a]	0.586±0.05[ab]	0.428±0.05[b]	0.0163
Leucine	1.185±0.08[a]	1.227±0.09[a]	0.708±0.10[b]	0.0041
Lysine	0.828±0.06[a]	0.635±0.07[b]	0.477±0.09[b]	0.0057
Methionine	0.292±0.03[a]	0.228±0.03[ab]	0.155±0.02[b]	0.0399
Phenylalanine	0.643±0.04[a]	0.586±0.04[a]	0.423±0.05[b]	0.0217
Threonine	0.588±0.04[a]	0.474±0.04[b]	0.360±0.05[b]	0.0076
Valine	0.894±0.06[a]	0.776±0.07[a]	0.535±0.07[b]	0.0230
Non-essential amino acids				
Alanine	0.980±0.07[a]	1.051±0.10[a]	0.477±0.06[b]	0.0071
Aspartic acid	1.387±0.08[a]	1.137±0.09[ab]	0.941±0.09[b]	0.0177
Cystine	0.062±0.01[a]	0.026±0.01[b]	0.022±0.00[b]	0.0241
Glucine	2.164±0.11	2.305±0.16	1.759±0.23	0.0995
Glycine	0.963±0.07[a]	0.671±0.07[b]	0.512±0.06[b]	0.0023
Proline	0.820±0.06	0.877±0.07	0.608±0.10	0.2110
Serine	0.852±0.07[a]	0.733±0.07[ab]	0.528±0.09[b]	0.0438
Tyrosine	0.555±0.05	0.534±0.05	0.410±0.07	0.5848
Total	14.494	13.099	9.467	

[abc row] Means with same letter are not different ($\underline{P} \geq 0.05$).

Table 4 Seasonal effects on amino acid concentrations of bobwhite crop contents. Amino acids expressed as a percent total amino acid ($\underline{X}$ ± $\underline{SE}$).

Amino acid	Season			
	May (n = 45)	September (n = 33)	February (n = 19)	($\underline{P}$ > F)
Essential amino acids				
Arginine	5.11±0.18^{b}	4.29±0.23^{c}	6.74±0.34^{a}	0.0001
Histidine	3.24±0.16	2.79±0.14	2.98±0.28	0.5970
Isoleucine	4.65±0.15	4.23±0.08	4.47±0.10	0.0834
Leucine	7.85±0.13^{b}	9.24±0.28^{a}	7.07±0.17^{c}	0.0001
Lysine	4.87±0.10^{a}	3.99±0.21^{b}	4.13±0.30^{b}	0.0001
Methionine	1.65±0.09	1.46±0.08	1.42±0.13	0.3242
Phenylalanine	3.43±0.07	3.45±0.08	3.55±0.10	0.9904
Threonine	4.34±0.07^{a}	3.84±0.05^{b}	4.06±0.09^{b}	0.0003
Valine	6.55±0.09^{a}	6.24±0.10^{b}	6.14±0.10^{b}	0.0355
Non-essential amino acids				
Alanine	9.42±0.21^{b}	11.25±0.34^{a}	7.27±0.22^{c}	0.0001
Aspartic acid	9.07±0.18^{b}	8.27±0.27^{c}	9.83±0.25^{a}	0.0010
Cystine	0.45±0.08	0.20±0.03	0.29±0.06	0.0525
Glucine	12.93±0.26^{b}	15.50±0.41^{a}	16.29±0.61^{a}	0.0001
Glycine	10.86±0.29^{a}	8.38±0.31^{c}	9.44±0.35^{b}	0.0001
Proline	6.08±0.13^{b}	7.37±0.15^{a}	5.90±0.40^{b}	0.0001
Serine	6.96±0.26	6.79±0.29	6.40±0.39	0.7844
Tyrosine	2.55±0.09^{b}	2.70±0.08^{ab}	3.01±0.36^{a}	0.0058
Total	100.01	99.99	99.99	

$^{abc\ row}$ Means with same letter are not different ($\underline{P} \geq 0.05$).

Table 5. Treatment effects on amino acid concentrations of bobwhite crop contents. Amino acids expressed on a dry weight basis ($\bar{X} \pm SE$).

	Treatment				
Amino acid	Herbicide (n = 14)	Herbicide + Fire (n = 28)	Mechanical (n = 18)	Control (n = 37)	($P > F$)
Crop protein, %	29.29±2.16	24.80±1.43	25.00±1.55	26.25±1.28	0.3168
Crop fat, %	13.59±1.55[b]	19.64±1.98[a]	7.40±0.85[c]	15.40±1.39[b]	0.0001
Essential amino acids					
Arginine	0.967±0.14	0.778±0.07	0.838±0.10	0.955±0.04	0.0799
Histidine	0.560±0.08	0.419±0.05	0.652±0.11	0.430±0.03	0.4414
Isoleucine	0.803±0.10	0.557±0.06	0.582±0.08	0.591±0.04	0.1715
Leucine	1.291±0.15	1.081±0.10	1.050±0.12	1.082±0.09	0.5915
Lysine	0.891±0.12[a]	0.602±0.07[b]	0.601±0.09[b]	0.733±0.07[ab]	0.0278
Methionine	0.307±0.06	0.229±0.04	0.283±0.05	0.211±0.02	0.9346
Phenylalanine	0.680±0.08	0.547±0.05	0.580±0.07	0.568±0.04	0.5670
Threonine	0.611±0.08	0.472±0.05	0.490±0.06	0.496±0.04	0.3599
Valine	0.997±0.13	0.727±0.07	0.734±0.10	0.770±0.06	0.1852
Non-essential amino acids					
Alanine	1.176±0.15	0.858±0.09	0.830±0.11	0.878±0.09	0.2769
Aspartic acid	1.413±0.17[a]	1.023±0.08[b]	1.164±0.13[ab]	1.274±0.09[ab]	0.0333
Cystine	0.072±0.03	0.041±0.01	0.055±0.02	0.025±0.00	0.7352
Glucine	2.260±0.22	1.979±0.16	1.859±0.17	2.279±0.16	0.1443
Glycine	1.014±0.14	0.707±0.08	0.718±0.12	0.746±0.06	0.1640
Proline	0.948±0.12	0.750±0.08	0.704±0.08	0.823±0.07	0.3055
Serine	0.884±0.12	0.725±0.08	0.628±0.08	0.765±0.08	0.4246
Tyrosine	0.632±0.10	0.435±0.06	0.502±0.07	0.549±0.05	0.1539
Total	15.515	11.930	12.270	12.175	

[abc row] Means with same letter are not different ($P \geq 0.05$).

Table 6. Treatment effects on amino acid concentrations of bobwhite crop contents. Amino acids expressed as a percent total amino acid ($\bar{X}$ ± SE).

	Treatment				
Amino acid	Herbicide (n = 14)	Herbicide + Fire (n = 28)	Mechanical (n = 18)	Control (n = 37)	(P > F)
Essential amino acids					
Arginine	4.34±0.24	4.87±0.32	5.10±0.32	5.70±0.26	0.2404
Histidine	2.84±0.18^{b}	2.82±0.14^{b}	4.05±0.28^{a}	2.78±0.16^{b}	0.0140
Isoleucine	4.95±0.44	4.41±0.09	4.46±0.15	4.35±0.08	0.2364
Leucine	7.98±0.37^{bc}	8.74±0.34^{a}	8.30±0.39^{ac}	7.75±0.12^{b}	0.0048
Lysine	4.99±0.28^{a}	4.05±0.20^{b}	3.92±0.23^{b}	4.69±0.18^{a}	0.0001
Methionine	1.60±0.12	1.56±0.13	1.89±0.16	1.34±0.06	0.1328
Phenylalanine	3.32±0.09^{b}	3.53±0.10^{ab}	3.64±0.11^{a}	3.38±0.06^{b}	0.0273
Threonine	4.10±0.13	4.16±0.10	4.29±0.11	4.00±0.06	0.5545
Valine	6.67±0.19	6.40±0.09	6.28±0.16	6.27±0.09	0.0729
Non-essential amino acids					
Alanine	10.67±0.42	9.96±0.39	9.50±0.41	9.03±0.38	0.2228
Aspartic acid	8.53±0.37	8.34±0.25	9.16±0.26	9.46±0.23	0.0635
Cystine	0.51±0.23	0.33±0.06	0.46±0.10	0.21±0.02	0.4771
Glucine	12.71±0.50^{b}	14.79±0.50^{ac}	13.62±0.50^{bc}	15.28±0.44^{a}	0.0460
Glycine	10.56±0.62	9.63±0.50	9.48±0.50	9.63±0.25	0.2218
Proline	6.66±0.28	6.79±0.24	6.43±0.31	6.73±0.20	0.2404
Serine	7.06±0.53	7.14±0.28	6.31±0.40	6.66±0.29	0.9872
Tyrosine	2.70±0.14^{ab}	2.42±0.13^{b}	2.91±0.23^{a}	2.79±0.16^{ab}	0.0108
Total	100.01	100.01	99.99	100.02	

abc row Means with same letter are not different ($P \geq 0.05$).

Table 7. Estimated dietary requirements of 10 essential amino acids for growth, reproduction, and maintenance (% dry weight).

Amino acid	Juvenile growth and Adult reproduction (NRC 1984)	Adult maintenance[a] (Eggum and Beams 1986)
Arginine	1.25	0.72
Histidine	0.36	0.30
Isoleucine	0.98	0.54
Leucine	1.69	0.84
Lysine	1.30	0.62
Methionine + Cystine	0.75	0.42
Phenylalanine	0.96	0.90
Threonine	1.02	0.48
Valine	0.95	0.60
Tryptophan	0.22	0.12

[a] Calculated using a winter maintenance of 12% protein (Nestler et al. 1944).

CHAPTER IV

THE USE OF CRUDE PROTEIN TO ASSESS DIET QUALITY: A REEXAMINATION WITH QUAIL

Abstract-- Nutritional factors have been hypothesized to regulate gallinaceous bird populations such as the bobwhite quail (Colinus virginianus). Although protein is considered one of the most important and limiting nutrient categories in wild animal populations, we lack a complete understanding of the availability of essential amino acids (EAA) in foodstuff protein. We provide evidence that crude protein (CP) may grossly over estimate true protein and provide a poor measure of biological value, the ability of protein to meet daily intake requirements for EAA. We investigated the availability of 17 amino acids in seed of four highly preferred forages of quail from a variety of habitat types in central Oklahoma. Although habitat type had no influence on the composition of EAA, mature seed from all species failed to meet the daily intake requirement of all 10 EAA. Deficiency of EAA in wooly croton (Croton capitatus) and sunflower (Helianthus annuus) were evident despite an apparently adequate

quantity of protein to meet recommended maintenance requirements of adults. The total nitrogen pool of seed was composed of 44-61% nonprotein nitrogen (NPN) of limited nutritional value. Computed biological values ranged from 58 (Paspalum floridanum) to 74 (Croton capitatus). Amino acid content of forages in lieu of CP may better describe the nutritional ecology of quail and other gallinaceous birds and provide new insights into the role of nutrition in regulating animal populations.

Nutrient limitations have been hypothesized as important factors in the regulation of many populations of gallinaceous birds, especially members of the Tetronidae and Phasianidae. Vitamin A, selected minerals such as phosphorus, and protein have been correlated with fluctuations in populations of gallinaceous birds (Nestler 1946; Schultz 1948; Lehmann 1953; Moss 1969, 1973; White 1978; Beckerton and Middleton 1982; Wood et al. 1986; Servello and Kirkpatrick 1987). Protein, in particular, is frequently limited in herbivore diets and deficiencies can suppress development, reproduction, and survival (Nestler et al. 1942; Carew and Hill 1961; Moss et al. 1975; Beckerton and Middleton 1982; Straznicka 1990). Gallinaceous birds are generally highly selective feeders, showing a preference for forages containing high concentrations of protein (Svoboda and Gullion 1972; Moss 1973; Doerr et al. 1974). Although protein is thought to be one of the most important

and limiting nutrients in wild populations, we unfortunately lack a complete understanding of its essential components, namely the availability of essential amino acids (EAA).

Dietary proteins are a diverse group of compounds composed of some 20 individual amino acids. Birds do not synthesize adequate quantities of about 10 amino acids (EAA) which must be consumed in the diet to meet daily requirements of these nutrients (Munks et al. 1946; Robbins 1983). Because proteins vary considerably in amino acid composition, they also vary greatly in their nutritive quality or biological value, the proportion of absorbed nitrogen retained for use by an animal (Erratum and Mitchell 1946; Oser 1959). As a result, crude protein (CP) estimates for diets may provide a poor index of protein quality (Sedinger 1984). Dietary deficiencies of an EAA may lead to reduced growth, reproductive performance, immunocompetence, or survival (Baldini et al. 1953; Okumura and Mori 1979; Robel 1979a; Allen and Young 1980; Begearmi et al. 1982; Harms and Buresh 1987; Tsiagbe et al. 1987; Klasing and Barns 1988).

Populations of many wild gallinaceous birds, including bobwhite quail (Colinus virginianus), are characterized by rather erratic and widely annual fluctuating populations (Lehmann 1953; Wood et al. 1986). Dietary requirements of 23-27% protein for optimum growth and reproduction (Baldini et al. 1950, Baldini et al. 1953, Tuttle et al. 1953, Andrews et al. 1973) and 11-12% protein for maintenance in

winter (Nestler et al. 1944) suggests that EAA deficiencies may be common given that seed are poor sources of EAA (Deyoe and Shellenberger 1965; VanEtten et al. 1967). Although considerable effort has been devoted to documenting availability of protein resources in the habitat of bobwhite quail, the biological value of these protein resources has been largely ignored. Sedinger (1984) noted that CP estimates of many common forages consumed by geese on tundra ranges over estimates true protein content by 22-52%, with major deficiencies in lysine, methionine, and cystine. Many agricultural cereal grains and legumes are deficient in multiple EAA despite a high concentration of CP (Deyoe and Shellenberger 1965; VanEtten et al. 1967; Hang et al. 1980).

High dietary requirements of bobwhite quail for EAA, coupled with strong evidence that crude protein may be a poor index of biological value in many forages, especially seed grains, led us to hypothesize that EAA resources are major limiting factors in bobwhite quail diets and that CP determinations may overestimate true protein content. We present evidence that CP is a poor indicator of true protein concentration of preferred wild seed consumed by quail. We show that a large component of the total nitrogen pool of these forages is often composed of nonprotein nitrogen (NPN) constituents of limited nutritional value and that concentrations of most EAA fail to meet daily requirements for maintenance. Our results indicate that a closer examination of the biological value of forages in lieu of CP

may better describe the nutritional ecology of this an other gallinaceous birds and provide new insights into the role of nutrition in regulating their populations.

METHODS

Seed grains comprise a major component of the annual diet of bobwhite quail throughout its geographic range (Bookhout 1958; Robel and Slade 1965). Quail show a distinct preference for seeds of wooly croton (Croton capitatus), common sunflower (Helianthus annuus), Florida paspalum (Paspalum floridanum), and western ragweed (Ambrosia psilostachia) in central Oklahoma (Baumgartner et al. 1952; Wiseman 1977; Rollins 1980; Tobler and Lewis 1981). We examined seed of these species for variation in the quality of protein by profiling concentrations of their respective amino acids in five disparate habitat types on the Cross Timbers Experimental Range (CTER), Payne County, Oklahoma (36°2' to 36°4'N, 97°9' to 97°11'W) located in the cross timbers land resource area (Garrison et al. 1977). Vegetation on CTER is similar to that of other areas in the cross timbers and is dominated by post oak (Quercus stellata) and black-jack oak (Quercus marilandica) in the overstory with interspersed tallgrass prairie (Ewing et al. 1984).

For each of the five habitat types, mature seed of each species were harvested from approximately 100 plants on each of four different 32-ha sample plots from 13 September to 25 October 1990. Seed of each species were pooled (n = 1)

within each habitat type prior to laboratory analysis. The five habitat types sampled included four habitats that were altered by removal of woody vegetation in the overstory and an unmodified control. The four altered habitats were: (1) mechanical brush removal, (2) herbicide application only, (3) herbicide application followed by annual burning, and (4) annual burning only. Removal of woody vegetation on altered habitats resulted in more grass and forb biomass compared to unmodified control sites; detailed descriptions of the study area have been previously published (Engle et al. 1991; Lochmiller et al. 1991; Stritzke et al. 1991).

Seed were separated from other plant material, cleaned by seiving, and composited by species and habitat type before further processing. Approximately 5 g samples were dried by lyophilization and ground to a fine powder using a micro-grinding mill. Fat was assessed by ether-extraction using a Soxhlet apparatus (Williams 1984). Total nitrogen pool was determined by micro-Kjeldahl analysis and converted to CP by multiplying the percent nitrogen by the correction factor 6.25.

Fat-extracted seed samples amounting to approximately 40 mg of protein were weighed into 25 X 150 mm glass tubes with teflon caps and hydrolyzed in 15 ml 6N HCL at 110 °C for 24 hr. One ml of the hydrolyzed sample was filtered through a 0.45-μm syringe filter (Acrodisc CRPTF, Fisher Scientific, Plano, Tex.). An internal standard (25 μl methionine sulfone) was added to 75 μl of filtered

hydrolysate before derivatization. Pre-column derivatization of amino acids was accomplished using phenylisothiocynate to produce phenylthiocarbamyl amino acids (Pico-Tag Workstation, Millipore Corporation, Milford, Mass.) and re-filtered through a 0.45-m syringe filter. Concentrations of 17 individual amino acids were determined using high pressure liquid chromatography (HPLC, Waters Model 820 system controller and Model 501 pumps). Tryptophan was destroyed by acid hydrolysis and therefore not measured. Chromatographic conditions were the following: Waters Pico-Tag Silica/C18 (15 cm by 3.9 mm) column; column temperature of 38 °C; flow rate of 1.0 ml/minute with pump back pressure of 5500 psi; system sensitivity of 489 mv/s (recorder) and 0.5 absorbance units full scale (Waters Model 484 UV detector, set at 254 nm); sample size of 4 μl; 28 minute run time. Solvents used were Eluent A and Eluent B (catalog number 88108 and 88112, respectively), Millipore Corporation (Milford, Mass.). Solvent conditions and gradients used for separation of amino acids were those described by Cohen et al. (1988). Amino acid concentrations were recorded as a relative proportion of the total amino acid pool and on a dry weight basis.

Non-protein nitrogen was assumed to be all nitrogen not incorporated into one of the 17 amino acids detected by HPLC (Bell 1963; Synge 1963). Nitrogen concentration of true protein in each sample was determined as the sum of all

amino acid nitrogen; NPN was calculated as the difference between total nitrogen (Kjeldahl analysis) and amino acid nitrogen (HPLC analysis).

A casein reference protein (from bovine milk, no. C-0376, Sigma Chem. Co.) of known amino acid composition was hydrolyzed and analyzed along with seed samples. Differences in percent fat, CP, NPN, and amino acid concentration among the five habitat types and among plant species were tested by analysis of variance (PROC ANOVA, SAS 1988). We used the Least Significant Differences test to isolate significant differences among means in the presence of a significant F-test ($P < 0.05$).

RESULTS

Crude protein, fat, and amino acid composition (expressed on a percent of total and dry weight basis) did not differ ($P > 0.05$) among the five habitat types. Variation in concentration of individual amino acids within a plant species was greater when expressed on a percent dry weight basis than relative percent of the total amino acid pool due to variation in percent crude protein (see SE in tables 1 and 2). Crude protein differed significantly ($P < 0.001$) among all plant species and ranged from 5.1 to 16.3% (Florida paspalum and wooly croton, respectively) (table 1). Differences were also noted for concentration of fat among plant species ($P < 0.001$); fat was lower in Florida paspalum than other species and lower in western ragweed than common sunflower.

All essential and nonessential amino acids differed ($P < 0.05$) among plant species when expressed as a relative percent of the total amino acid pool (table 1). This indicated that the quality of proteins as measured by the EAA composition varied considerably among species. Differences in amino acids among plant species when expressed on a dry weight basis were less apparent (table 2).

Comparisons of the total nitrogen pool (Kjeldahl analysis) to the amino acid nitrogen pool (HPLC analysis) reveal that a substantial quantity of nitrogen was incorporated into NPN constituents of limited nutritional value (table 3). Mean NPN values ranged from 44.31% for western ragweed to 61.34% for common sunflower, but were not different ($P > 0.05$) among habitat types or plant species. Crude protein concentrations adjusted for NPN yielded estimates of true protein well below CP. The calculated conversion factor for adjusting Kjeldahl nitrogen values to reflect true protein concentration averaged 2.98 compared with the standard of 6.25.

Dietary requirements of EAA for Japanese quail (*Coturnix coturnix*) and bobwhite quail have been published (National Research Council 1984; Eggum and Beams 1986) and are routinely reported as a percent dry weight of diet (table 4). A comparison of the concentrations of each EAA in the four wild seed grains (table 2) to dietary requirements for growth and reproduction showed all

nutrients to be limiting, regardless of crude protein content of plants. The sulfur-containing amino acid methionine (plus cystine) was low in all species; deficiencies ranged from 81.1 to 96.0% (100 x (requirement - available)/requirement). Lysine and leucine concentrations also were consistently low; deficiencies ranged from 70.0 to 89.5%. Concentrations of valine and arginine came closest to meeting the daily intake requirements of quail; deficiencies ranged from 22.7 to 86.6%.

The overall adequacy of a protein food source for supporting maintenance, growth, and reproductive demands of a bird will be dependent upon how well the proteins supply the relative needs for EAA (Oser 1959). In practice, determining how well a protein meets the daily intake requirements for a specific amino acid depends upon the relative supply of all other amino acids. Several empirical techniques have been developed to evaluate the overall nutritional quality or biological value of proteins based upon their respective EAA profiles. Examples include the use of chemical scores based on the amount of the EAA in greatest deficit in a protein (Mitchell and Block 1946) and the Essential Amino Acid Index (EAAI) which compares the ratios of EAA in a protein relative to their respective amounts in a high quality reference protein such as whole egg protein or casein (Oser 1959). Oser (1959) further documented a strong regression relationship between the EAAI and biological value of a protein.

We calculated the chemical score, EAAI, and biological value as described above for all wild seed grains (table 5). The chemical score, EAAI, and biological value differed significantly ($P \geq 0.001$) between wooly croton and the other seed species; however, differences between western ragweed and common sunflower were not apparent. All measures of overall nutritional quality of seed proteins resulted in similar rankings, with wooly croton most closely approaching the requirements of quail. Empirically derived values of biological value using Oser's (1959) regression against computed EAAI yielded a range from 55 for Florida paspalum to a high of 71 for wooly croton. Two of the most widely used seed grains of quail in central Oklahoma and Kansas, western ragweed and common sunflower (Robel and Slade 1965), had estimated biological values of only 59.

DISCUSSION

White (1978) proposed that available nitrogenous nutrients were the most limiting environmental resource to wild herbivore populations. Scarce quantities of digestible protein in plant material, high nitrogen requirements for reproduction, differential forage selectivity by individuals, and high juvenile mortality rates were offered as strong supporting evidence by White (1978). Bobwhite quail populations in the Midwest exhibit erratic annual fluctuations in density (Roseberry and Klimstra 1984) and individuals have a high dietary requirement of protein (23%) for growth and reproduction (Nestler et al. 1942). Hurst

(1972) hypothesized that protein shortages may be a primary factor regulating chick survival and winter is considered to be a major period of nutritional stress for adult birds (Robel 1965; Robel and Fretwell 1970). The long-term consequences of protein malnutrition during winter and early spring on subsequent reproduction are unknown for quail, but studies with red grouse (Lagopus seotieus) suggest that a strong relationship could exist (Moss 1973).

Considerable efforts have been devoted to documenting CP in foods (Nestler et al. 1945; Newlon et al. 1964) and diets (crop contents; Wood et al. 1986) of bobwhite quail. Surprisingly, no attempts to document true protein concentration or biological value of food proteins and diets of quail or other gallinaceous birds exist to our knowledge. Sedinger's (1984) analysis of amino acid concentrations in a variety of tundra plants used by geese indicated that CP determinations can overestimate true protein content by 22-52%. We hypothesized that similar discrepancies existed with foods utilized by bobwhite quail, especially, given the high concentrations of NPN constituents found in many cultivated and weed seed grains (VanEtten et al. 1967; Holt and Sosulski 1981). We choose to examine the quality of protein in seed from four ubiquitous plant species that are highly preferred and consumed in large amounts by bobwhite quail (Wood et al. 1986).

Significance of Nonprotein Nitrogen

Nonprotein nitrogen constituents were found to be significant components of the total nitrogen pool in all four seed grains. Nonprotein nitrogen sources are a highly varied group of compounds in plants and include alkaloids, ammonium salts, nitrogenous glucosides and lipids, amides, purine and pyrimidine compounds, nitrates, urea, nucleosides and nucleotides, porphyrins, betaines and a variety of free amino acids such as α-aminobutyric and α-aminoadipic acid (Maynard et al. 1979, Holt and Sosulski 1981, Singh and Jambunathan 1981, Oka and Sasaoka 1985). A variety of definitions exist for NPN (Holt and Sosulski 1981); we defined NPN as all nitrogen not incorporated (bound to protein or free amino acids) into one of the 17 amino acids detected by HPLC.

The nutritive value of these nonprotein nitrogen-containing compounds to non-ruminant animals is not entirely clear. Traditionally, nutritionist have used the rule-of-thumb of assigning half the nutritional value of protein nitrogen to NPN (Synge 1963). However, this is inconsistent because it apparently assumes the presence of nutritionally relevant free amino acids such as glycine, alanine, serine, glutamine, and others in the NPN category. We did not include these free amino acids as part of the nonprotein nitrogen pool since they were measured (HPLC) as part of the total amino acid pool.

Conversion of Kjeldahl nitrogen to CP based on the traditional conversion factor of 6.25 appears to greatly overestimate protein quality and overall biological value of wild seed grains. The average conversion factor for adjusting Kjeldahl nitrogen determinations to reflect true protein concentration in our study was 2.98.

Digestibility of dietary protein is another important factor that can drastically influence protein requirements and the ability of certain protein sources to meet these requirements (Owens and Pettigrew 1989). Digestibility of seed from many wild plant species used by bobwhite quail range from 40 to 90% (Robel et al. 1979b, 1979c). Both nonprotein nitrogen and protein digestibility undoubtedly act in concert to reduce the overall nutritional value of nitrogen and distorts the usefulness of CP as a measure for determining the adequacy of foods in meeting daily protein requirements.

A solution to the problems associated with assessing protein quality in diets is to compare EAA composition of foods to dietary requirements. Dietary protein and EAA requirements are usually determined experimentally using purified protein sources to formulate rations (with amino acid supplements) with a known EAA composition (Allen and Young 1980; Serafin 1982). Nonprotein nitrogen composition in purified protein sources, such as isolated soybean meal protein or casein, would normally be low compared to the whole food source (Bell 1963). Given the large and highly

variable concentrations of NPN in wild seed grains compared to purified protein sources and specific dietary requirements for EAA, profiles of concentrations of EAA would provide a more accurate determination of forage quality.

Influence of Environment on Protein Quality

Although all measures of nutritive quality of wild seed grains were variable to some degree both within and among species, habitat type did not appear to account for the observed variation, especially when expressed relative to the total amino acid pool. It has been well documented that gross measures of nutritive quality such as CP (Mangaroo 1984; Rendig 1984; Szuts et al. 1988; Flower et al. 1989), soluble carbohydrates (Anderson et al. 1989; Benzing-Purdie and Nikiforuk 1989), and crude fat (Stark 1924) in plants reflect differences in soil fertility. Studies examining whether amino acid composition of specific plant proteins are similarly influenced by fertility or other environmental traits have been contradictory. Robinson (1975) provided strong evidence that environment can alter the relative proportions of amino acids in various kinds of protein in cultivated sunflower seeds. In contrast, other studies elucidating the effects of nitrogen fertilization on amino acid composition of various cultivated crops have not documented any significant alterations in amino acid profiles (Eppendorfer 1977; Meredith and Gaskins 1984; Meredith et al. 1984).

Are Essential Amino Acid Deficiencies Common?

All seed species fell well short of meeting the EAA requirements of bobwhite quail for growth and reproduction. No EAA was concentrated enough in the four highly preferred seed species analyzed in our study to meet daily intake requirements of quail according to National Research Council (1984) guidelines. Tryptophan, an EAA comprising about <1.0% of the total dry weight of seeds (Harrold and Nalewaja 1977), was not measured in seeds because it is destroyed by acid hydrolysis. Some loss (about 15%) of methionine and cystine undoubtedly occurred as well due to varying degrees of destruction in acid hydrolysis (Spindler et al. 1984; Elkin and Griffith 1985). The sulfur-containing EAA methionine and cystine were the most limiting amino acids, even considering analytical inaccuracies. Likewise, lysine was extremely deficient in all seeds. Among all EAA, valine and arginine came closest to meeting growth and reproductive requirements but were still 22-86% deficient. Considering that published protein requirements for quail chicks is about 24% (Serafin 1977), all four seed species were deficient.

Protein requirements of quail hens in early spring (leading up to the breeding season) for optimum performance during the egg-laying season are unknown. However, winter maintenance requirements for protein in the adult bird have been estimated at about 12% (Nestler et al. 1944). Both wooly croton (16.3%) and common sunflower (13.8%) appeared

to contain suitable concentrations of CP to meet winter maintenance requirements. Comparison of adult maintenance requirements for EAA in table 4 to levels of EAA in these two seed grains (table 2) revealed deficiencies for all EAA in common sunflower (ranging from 25 to 85% deficient) and all but one (arginine) EAA in wooly croton (ranging from 0 to 70% deficient). Overall, EAA deficiencies averaged 36.0% in wooly croton and 50.9% in common sunflower, despite apparently adequate concentrations of crude protein.

These results highlight important concerns in the use of measures such as CP to assess the nutritional quality of common bobwhite quail foods, as well as foods of other qallinaceous birds. Because animals have dietary requirements for individual amino acids rather than protein, protein estimates should probably be used as an index of nutritive quality only and not used to assess the ability of particular forages or diets to meet nutrient intake requirements of birds. Failure of previous studies (Roseberry and Klimstra 1984) to find any significant relationships between dietary protein estimates and various intrinsic characteristics of populations (e.g., density, survival rates, recruitment rates) could have been due in part to the inherent inaccuracies associated with measures of CP.

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TABLE 1
Mean (±SE) percent fat, crude protein, and amino acid composition of wooly croton, Florida paspalum, western ragweed, common sunflower, and casein. Amino acids expressed as a percent of total amino acid.

Amino Acid	Croton (n = 5)	Paspalum (n = 5)	Ragweed (n = 5)	Sunflower (n = 5)	Casein[1] (n = 1)
% Fat	18.08±2.83^{ab}	0.60±0.19^{c}	14.73±1.20^{b}	20.14±1.19^{a}	---
% Crude Protein	16.27±0.58^{a}	5.07±0.29^{d}	9.17±0.72^{c}	13.83±0.81^{b}	83.00
Essential Amino Acids					
Arginine	9.45±0.21^{a}	3.75±0.42^{c}	6.71±0.13^{b}	6.27±0.16^{b}	3.86
Histidine	2.57±0.11^{a}	1.60±0.04^{c}	2.04±0.04^{b}	1.88±0.02^{b}	2.58
Isoleucine	4.95±0.05^{a}	4.32±0.08^{b}	4.77±0.11^{a}	4.93±0.03^{a}	5.08
Leucine	6.34±0.04^{c}	8.90±0.22^{a}	7.07±0.03^{b}	6.80±0.01^{b}	8.71
Lysine	4.79±0.27^{a}	3.61±0.34^{b}	4.38±0.14^{ab}	4.58±0.09^{a}	8.09
Methionine	1.13±0.17^{a}	0.62±0.07^{b}	0.47±0.05^{b}	0.73±0.05^{b}	2.48
Phenylalanine	4.09±0.04^{b}	5.26±0.32^{a}	3.85±0.02^{b}	3.88±0.03^{b}	4.57
Threonine	3.73±0.15^{b}	4.28±0.08^{a}	4.10±0.08^{a}	3.68±0.08^{b}	5.01
Valine	7.26±0.12^{a}	6.42±0.14^{b}	5.90±0.12^{c}	6.56±0.06^{b}	6.48
Non-Essential Amino Acids					
Alanine	6.94±0.07^{b}	12.94±0.36^{a}	6.81±0.12^{b}	6.76±0.12^{b}	3.45
Aspartic acid	11.10±0.24^{a}	9.00±0.57^{b}	10.77±0.22^{a}	10.12±0.22^{ab}	8.10
Cystine	0.25±0.07^{a}	0.09±0.01^{b}	0.24±0.02^{a}	0.11±0.01^{b}	0.00
Glucine	14.71±0.23^{b}	15.55±0.76^{b}	19.31±0.50^{a}	18.85±0.47^{a}	20.38
Glycine	10.68±0.38^{ab}	7.21±0.51^{c}	9.47±0.19^{b}	11.92±0.33^{a}	2.49
Proline	5.65±0.08^{c}	8.18±0.25^{a}	6.49±0.20^{b}	6.30±0.08^{b}	9.56
Serine	3.87±0.36^{b}	5.82±0.24^{a}	6.09±0.20^{a}	4.46±0.28^{b}	5.23
Tyrosine	2.49±0.11^{a}	2.46±0.08^{ab}	1.54±0.06^{c}	2.17±0.12^{b}	5.01
Total	100.00	100.00	100.00	100.00	101.80

abcd row Means with different letters are different ($P \geq 0.05$).

[1] Reference protein.

TABLE 2

Mean (±SE) amino acid composition of wooly croton, Florida paspalum, western ragweed, common sunflower, and casein. Amino acids expressed as a percent of dry weight basis.

Amino Acid	Croton (n = 5)	Paspalum (n = 5)	Ragweed (n = 5)	Sunflower (n = 5)	Casein[1] (n = 1)
Essential Amino Acids					
Arginine	0.966±0.214^{a}	0.168±0.016^{b}	0.519±0.048^{b}	0.539±0.082^{b}	0.281
Histidine	0.230±0.044^{a}	0.065±0.007^{b}	0.140±0.011^{b}	0.142±0.019^{b}	0.174
Isoleucine	0.374±0.072^{a}	0.148±0.012^{b}	0.275±0.019^{ab}	0.315±0.042^{a}	0.344
Leucine	0.479±0.092^{ns}	0.307±0.031	0.410±0.031	0.436±0.060	0.689
Lysine	0.390±0.058^{a}	0.137±0.012^{b}	0.282±0.020^{a}	0.325±0.040^{a}	0.648
Methionine	0.106±0.033^{a}	0.024±0.003^{b}	0.031±0.003^{b}	0.053±0.009^{ab}	0.168
Phenylalanine	0.390±0.077^{ns}	0.230±0.030	0.281±0.022	0.314±0.045	0.308
Threonine	0.260±0.056^{ns}	0.134±0.011	0.215±0.014	0.215±0.032	0.288
Valine.	0.495±0.104^{a}	0.197±0.016^{b}	0.305±0.022^{ab}	0.374±0.049^{ab}	0.439
Non-Essential Amino Acids					
Alanine	0.356±0.068^{ns}	0.300±0.029	0.268±0.018	0.295±0.041	0.234
Aspartic acid	0.843±0.154^{a}	0.310±0.018^{b}	0.633±0.048^{a}	0.651±0.078^{a}	0.649
Cystine	0.036±0.007^{a}	0.006±0.001^{b}	0.026±0.002^{ab}	0.014±0.001^{b}	0.00
Glucine	1.258±0.263^{a}	0.605±0.070^{b}	1.265±0.126^{a}	1.371±0.211^{a}	1.378
Glycine	0.455±0.080^{a}	0.140±0.010^{b}	0.313±0.021^{a}	0.431±0.049^{a}	0.169
Proline	0.373±0.070^{ns}	0.248±0.025	0.329±0.025	0.355±0.049	0.649
Serine	0.248±0.068^{ns}	0.161±0.017	0.282±0.022	0.235±0.043	0.364
Tyrosine	0.260±0.051^{a}	0.118±0.013^{b}	0.124±0.013^{b}	0.187±0.018^{ab}	0.338
Total	7.52	3.30	5.70	6.26	7.12

$^{ab\ row}$ Means with different letters are different ($P \geq 0.05$).

^{ns} Not significant ($P > 0.05$).

[1] Reference protein.

TABLE 3

Measure of total nitrogen (determined by Kjeldhal analysis) and amino nitrogen (HPLC amino acid analysis) in 4 important seed grains in the diet of bobwhite quail. The percentage of the total nitrogen pool recovered as amino nitrogen was calculated by difference and reported as non-protein nitrogen (% NPN). Correction factors for converting total nitrogen determinations (by kjeldhal analysis) to protein estimates are provided.

Plant	Kjeldhal Nitrogen (mg N/g DW)	Amino Acid Nitrogen (mg N/g DW)	NPN (%)	Correction Factor	Adjusted CP
Croton	26.04±0.92	11.22±2.25	57.13±8.18	2.68	7.02±1.41
Paspalum	8.11±0.46	4.33±0.34	46.65±2.52	3.33	2.71±0.21
Ragweed	14.67±1.15	7.97±0.63	44.31±6.38	3.48	4.98±0.39
Sunflower	22.12±1.30	8.72±1.21	61.34±3.34	2.42	5.45±0.75

TABLE 4
Estimated dietary requirements of 10 essential amino acid for growth in quail, rabbit, and rat (% dry weight).

Amino Acid	Quail		Rabbit	Rat
	Juvenile growth (NRC 1984)	Adult maintenance[a] (Eggum and Beams 1986)	(NRC 1977)	(NRC 1978)
Arginine	1.25	0.72	0.60	0.67
Histidine	0.36	0.30	0.30	0.33
Isoleucine	0.98	0.54	0.60	0.61
Leucine	1.69	0.84	1.10	0.83
Lysine	1.30	0.62	0.65	1.00
Methionine+ Cystine	0.75	0.42	0.60	0.67
Phenylalanine	0.96	0.90	1.10	0.89
Threonine	1.02	0.48	0.60	0.56
Valine	0.95	0.60	0.70	0.67
Tryptophan	0.22	0.12	0.20	0.17

[a] Calculated using a winter maintenance of 12% protein (Nestler et al. 1944).

TABLE 5
Percent protein, essential amino acid index (EAAI), calculated biological value (CBV), first limiting amino acid (FLAA), and calculated chemical score (CCS) of 4 seed species.

Seed	% Protein	EAAI[a]	CBV[b]	FLAA	CCS[c]
Croton	16.27	76	71	Methionine	36
Paspalum	5.07	61	55	Methionine	20
Ragweed	9.17	65	59	Methionine	15
Sunflower	13.83	65	59	Methionine	23
Casein[1]	83.00	89	85	---	--

[a] EAAI = $\sqrt[n]{\frac{Arg_p}{Arg_s} \times \frac{His_p}{His_s} \times \ldots \frac{Val_p}{Val_s}}$ in which the subscript p refers to the food protein; s, the whole egg protein; and n, the number of amino acids (counting methionine and cystine as one). The 9 essential amino acids included in all computations are arginine, histidine, isoleucine, leucine, lysine, methionine + cysteine, phenylalanine, threonine, and valine (Oser 1959).

[b] Computed biological value = 1.09(EAAI)-11.7 (Oser 1959).

[c] Computed chemical score = the percentage deficient in the limiting EAA subtracted from 100 (Mitchell and Block 1946).

[1] Reference protein.

CHAPTER V

PROFILES OF SERUM AMINO ACIDS TO ASSESS CONDITION OF COTTON RATS (SIGMODON HISPIDUS)

ABSTRACT.--We investigated the use of concentrations of blood serum amino acids to assess dietary protein status of juvenile cotton rats (Sigmodon hispidus). Eighteen juvenile animals were randomly assigned to isocaloric diets containing either 4 or 16% crude protein and serum concentrations of amino acids were determined by high pressure liquid chromatography on days 30 and 45 of a 45-day feeding trial. Concentrations of methionine, threonine, tryptophan, valine, hydroxyproline, and branch chain amino acids (BCAA) were depressed while histidine and proline were elevated among low-protein fed cotton rats. The glycine/(leucine + valine) and phenylalanine/tyrosine ratios were greater in serum of cotton rats fed low-protein diet while the tyrosine/neutral amino acids (NAA) and tryptophan/NAA ratios were depressed. Concentration of several free amino acids in serum of juvenile cotton rats differed between 30- and 45-day samples. Because diet X sample

interactions were significant for only tryptophan and hydroxyproline, temporal changes were probably a reflection of age-related alterations in developmental physiology and not effects of diet. Our results indicate that clinical profiles of selected amino acids in serum can provide a useful technique for assessing protein nutritional status and diet quality in small mammals.

Protein malnutrition is probably a common occurrence in wild herbivore populations, influencing the dynamics of many populations through depressed reproduction and survival of the young (Belovsky, 1986; Hansson, 1979; Moss, 1973; White, 1978). Several methods have been proposed to ascertain the protein nutritional status of small mammals or indirectly assess quality of their habitat. Body condition indices such as weight/length ratios, body fat, and lean mass have been used widely to assess the general condition of small mammals (Cengel et al., 1978; Lochmiller et al., 1983; Merson and Kirkpatrick, 1981). Although gross measures of morphology and composition of body tissue are useful indicators of an animal's general nutritional history, short-term alterations in diet quality or animal condition are not easily detected by these approaches. Physiological characteristics of animals, such as concentrations of selected metabolites in blood serum, tend to be more responsive to acute changes in nutritional status. Consequently, several techniques that use concentrations of

various cellular components, proteins, enzymes, and metabolites in blood to assess nutritional status have been examined in a variety of animal species (Florence et al., 1983; Hackbarth et al., 1983; Lakshmanan et al., 1983; Sarisnerand et al., 1990; Warren and Kirkpatrick, 1978).

Profiles of concentrations of free amino acids in blood serum have been used in the diagnosis of subclinical protein malnutrition in humans (Antener et al., 1981a, 1981b; Johnson and Anderson, 1982; McLaren et al., 1965) and have been suggested as the most sensitive indicator of protein nutritional status of laboratory rats (Gustafson et al., 1986; Jansen et al., 1986). Protein malnutrition alters serum amino acid patterns due to decreased amino acid synthesis (Elwyn, 1988), increased muscle catabolism (Grimble and Whitehead, 1970a, 1970b), and decreased amino acid absorption (Young and Marchini, 1990). Clinical evaluations incorporating concentrations of free amino acids in blood may provide a more sensitive assessment of nutritional status in wild small mammals than traditional body condition indices (Gustafson et al., 1986; Johnson and Anderson, 1982; Seal et al., 1978, Tsuda et al., 1989).

Our primary objective was to evaluate the feasibility of using physiological profiles of the free amino acid pool in serum to assess protein nutritional status of cotton rats (*Sigmodon hispidus*). We documented changes in concentrations of both essential and non-essential amino

acids in sera of juvenile cotton rats fed either a high or low protein diet.

MATERIALS AND METHODS

Experimental Subjects and Design

Juvenile cotton rats (30 days old) used in this study were offspring of wild-caught parents maintained in an outbred laboratory colony under room temperature and 14L:10D conditions. Animals were fed a commercial laboratory ration (Purina, St. Louis, MO) before their use in experimental feeding trials. Eighteen juveniles were weighed (initial body weight) and randomly assigned to one of two isocaloric rations formulated to provide either a high (16%) or low (4%) concentration of protein (Table 1). Animals were paired within sexes, housed in hanging wire-bottom cages (24 by 18 by 18 cm), and fed their respective experimental diets ad libitum for 45 days.

Body weights were obtained and blood samples collected on days 30 and 45 of the feeding trial. Animals were anesthetized with an intramuscular injection of ketamine hydrochloride (Bristol Laboratories, Syracuse, NY) at 50 mg/kg body weight prior to bleeding. Blood was collected from the retro-orbital sinus plexus into 10-ml serum separation tubes and centrifuged at 1500 x g for 10 min at 15 °C. Serum was harvested and stored frozen at -20 °C until free amino acid analysis could be performed.

Amino Acid Analysis

Serum (1 ml) from each animal was deproteinized by filtering through a 10,000 molecular weight (μm) cut-off ultrafiltration membrane filter (Ultrafree-MC, Millipore, Milford, MA) by centrifugation at 1,000 x g for 15 min. An internal standard (25 μl methionine sulfone) was added to 75 μl of filtered serum prior to derivatization. Pre-column derivatization of free amino acids was accomplished using phenylisothiocynate to produce phenylthiocarbamyl amino acids (Pico-Tag Workstation; Millipore) and re-filtered through a 0.45-μm syringe filter (Acrodisc CRPTFC, Fisher Scientific, Plano, TX). Concentrations of 38 individual amino acids were determined in derivatized serum samples using high pressure liquid chromatography (Waters Model 820 system controller and Model 501 pumps, Millipore). Arginine was not included in analysis due to difficulties in peak separation. The following chromatographic conditions were used: Waters Pico-Tag silica/C18 (30 cm by 3.9 mm) column; column temperature of 46 °C; flow rate of 1.0 ml/min with back pressure of 5500 psi; system sensitivity of 489 mv/s (recorder) and 0.5 absorbance units full scale (Waters Model 484 UV detector, set at 254 nm); sample size of 10 μl; and 87 min run time. Solvent conditions and gradients used for separation of amino acids were those described by Cohen et al. (1988). Amino acid concentrations were recorded as μmol/dl.

Statistical Analysis

The statistical significance ($P \leq 0.050$) of differences between the means of dietary treatments (high or low protein) were determined using two-way analysis of variance with repeated measures (PROC ANOVA; SAS, 1988). An unbalanced data set resulted from our inability to obtain a sufficient volume of serum from three animals fed the low protein diet. The statistical significance of the mean of the differences due to duration of trial (30 or 45 days; 4 and 16% data sets were combined within each sample time) were determined using a paired t-test to account for repeated measures. Statistical interactions between diet treatment (high or low protein) and duration of trial (30 or 45 days) was determined using an unpaired t-test on the difference of the differences due to trial duration.

RESULTS

Initial mean body weights of juvenile cotton rats on the high (32.9 ± 1.6 SE g) and low (32.8 ± 3.3 g) protein diets were not significantly different. Rate of body growth as measured by percent change in body mass during the trial differed significantly ($P < 0.001$) between dietary protein groups. High protein-fed cotton rats developed normally with an overall mean body weight change of 148.1 ± 13.0%. Those individuals on the low protein diet were able to maintain initial body weight but demonstrated no appreciable growth (-0.2 ± 2.7%). Cotton rats fed a high protein diet gained an average of 47.5 g.

Amino Acid Profiles

Twenty-four amino acids occurred at sufficient concentrations in serum of cotton rats for detection by the methodology we used in this study (Table 2). Alanine and glycine were two of the most concentrated amino acids in serum of cotton rats; concentrations of 1-methylhistidine and 3-methylhistidine were extremely low and detected in only a few individuals.

Total concentrations of branch chain (BCAA = leucine + isoleucine + valine), neutral (NAA = leucine + isoleucine + valine + tyrosine + phenylalanine + tryptophan), aromatic (AROM = phenylalanine + tyrosine + tryptophan), and sulfur-containing amino acids (SAA = methionine + cysteine) were significantly ($P = 0.018$, $P = 0.002$, $P < 0.001$, $P = 0.034$, respectively) higher in high than low protein-fed animals (Fig. 1). Total concentration of essential amino acids (EAA) in serum tended to be ($P = 0.066$) greater among those fed a high protein diet than a low protein diet (Fig. 1); differences in total concentration of non-essential amino acids (NEAA) were not significant ($P > 0.01$).
The essential/non-essential (EAA/NEAA) ratio, as calculated by Gustafson et al. (1986), tended ($P = 0.074$) to be higher in the 16% than 4% protein group (Fig. 1).

With the exception of histidine, concentrations of all essential amino acids were higher in juveniles fed a high protein diet compared to low protein (Table 2; mean of 30- and 45-day sample); histidine was significantly ($P = 0.006$)

higher in the low protein group. Differences in mean concentrations between high and low protein-fed juveniles were significant for the essential amino acids valine (P = 0.003), threonine (P = 0.028), methionine (P = 0.007), and tryptophan (P = 0.001). There was a significant (P < 0.001) interaction between diet and sample for tryptophan, which was higher in the high protein than low protein group on day 45.

Very few of the non-essential amino acids differed in concentration between dietary treatments. Concentrations of tyrosine, hydroxyproline, and serine (P < 0.001, P = 0.035, and P = 0.015, respectively) were higher in the high than low protein group (Table 2). There was a significant (P = 0.045) interaction between diet and sample for hydroxyproline; hydroxyproline concentration was higher in the high protein than low protein group at 45 days.

Ratios of phenylalanine/tyrosine (P < 0.001) and glycine/(leucine + valine) were significantly (P < 0.001) lower in animals fed a high protein diet than those on low protein rations (Fig. 1). Elevated concentrations of tyrosine and tryptophan after 45 days contributed to significantly higher tyrosine/NAA (P < 0.001) and tryptophan/NAA (P < 0.001) ratios in the high than low protein diet group.

Temporal Changes in Amino Acid Profiles

In general, free amino acid profiles of juvenile cotton rats were very dynamic, as several significant changes were

observed between 30- and 45-day samples (Fig. 1). Total BCAA, NAA, and NEAA pools were significantly greater (P = 0.002, P = 0.047, and P = 0.002, respectively) at 30 days compared to 45 days; while AROM amino acids (P = 0.004) were depressed. Concentrations of seven of the essential amino acids differed significantly between samples. Histidine, lysine, phenylalanine, and tryptophan concentrations were greater (P = 0.004, P = 0.002, P = 0.003, and P < 0.001, respectively) while concentrations of isoleucine, methionine, and valine were lower (P < 0.001, P < 0.001, and P = 0.038, respectively) at 45 days compared to 30 days (Table 2). Several nonessential amino acids also differed significantly with sample. Concentrations of alanine, proline, and tyrosine were lower (P < 0.001, P = 0.007, and P = 0.019, respectively) while citrulline, cysteine, and ornithine were greater (P < 0.001) at 45 days compared to 30 days.

Temporal changes also were noted in several descriptive ratios of the free amino acid pool (Fig. 1). The ratios of phenylalanine/tyrosine, EAA/NEAA, and tryptophan/NAA (P < 0.001, P = 0.003, and P < 0.001, respectively) were greater and the tyrosine/NAA ratio was lower (P = 0.017) at 45 days compared to 30 days.

DISCUSSION

Concentrations of free amino acids in serum and tissues represent an equilibrium between amino acid intake in food, rate of use in protein synthesis, and muscle catabolism or

amino acid oxidation (Galibois et al., 1987; Larbier et al. 1982). As intake of protein, and to some degree energy, is modified the rates of these physiologic systems which are responsible for maintaining amino acid homeostasis can be expected to change in an animal (Young and Marchini, 1990). Clinical approaches are readily available for detecting alterations in amino acid homeostasis in human medicine (Gibson, 1990). The ease of obtaining blood samples in the field and the availability of commercial blood testing laboratories and test kits makes similar clinical approaches to assessing protein nutritional status in wild rodents such as the cotton rat attractive as well.

During protein malnutrition or negative nitrogen balance the body often attempts to conserve nitrogen and EAA. Previous studies with laboratory rodents have indicated that as dietary protein levels decrease, serum free EAA decrease whereas the NEAA, especially glycine, tend to remain normal or increase (Fashakin and Furst, 1987; Harper et al., 1970; McLaughlan and Illman, 1967; Peng et al., 1970). In general, a similar trend was evident in our study where concentrations of the EAA methionine, threonine, tryptophan, and valine were depressed and the NEAA proline was elevated among low-protein fed cotton rats. The glycine/(leucine + valine) ratio has been used as an abbreviated method for determining the EAA/NEAA ratio in human medicine (Gibson, 1990). Although the EAA/NEAA ratio did not differ between diet groups in this study, the

glycine/(leucine + valine) ratio was greater in serum of cotton rats fed low protein due to a tendency for lower concentrations of leucine. When animals are subjected to a low protein diet, the observed decrease in EAA and concomitant increase in NEAA may be partly explained by their inability to synthesize EAA but synthesize NEAA as they are utilized (Peng and Harper, 1970; Whitehead and Dean, 1964), often resulting in lower EAA/NEAA or glycine/(leucine + valine) ratios.

Other amino acids such as the BCAA (leucine, isoleucine, valine) have been shown to be linearly related to intake (Johnson and Anderson, 1982; Peters and Harper, 1985) and have been useful as an index for the diagnosis of malnutrition in humans (Gibson, 1990). In addition to valine, concentrations of leucine in the cotton rat tended to support this observation.

Several studies have indicated that as dietary protein levels decrease, concentrations of serum histidine, an essential amino acid for growth, increase in laboratory rats (Fashakin and Furst, 1987; Peters and Harper, 1985). Histidine was the only serum EAA in cotton rats that was elevated in the low protein group. Jansen et al. (1986) indicated that increased histidine concentrations during protein malnutrition may be due directly to an increase in muscle protein catabolism. However, decreased catabolism or increased transport of histidine in the protein-deficient state may also be involved (Gustafson et al., 1986).

Histidine has been identified as a neurotransmitter precursor and its elevation may act as an endogenous signal of protein intake (Enwonwu and Wothington, 1974).

Hydroxyproline concentrations were depressed in cotton rats fed the low protein diet. Hydroxyproline is found predominantly in collagen which is associated with bone cartilage and connective tissues (Jones et al., 1990; Nguyen and Zarkadas, 1989). Low protein diets suppressed growth rates of cotton rats which could have altered the turnover of collagen in bone and cartilage resulting in depressed serum concentrations.

The ratios of tryptophan and tyrosine to the total NAA pool declined in low protein-fed cotton rats as result of greater reductions in the concentrations of these two NAA relative to the total NAA pool. Gustafson et al. (1986) found that in laboratory rats the tryptophan/NAA ratio relates to protein intake and increases with an increase in intake of crude protein. Elevations in tryptophan, a precursor of the neurotransmitter serotonine, and the tryptophan/NAA ratio are thought to be associated with appetite suppression (Tsuda et al., 1989). Depressed tryptophan/NAA ratios among protein restricted cotton rats may be a metabolic adaptation for increased food intake. Phenylalanine hydroxylase activity often declines during malnutrition resulting in a decline in the rate of conversion of phenylalanine to tyrosine (Gibson, 1990). Elevated phenylalanine/tyrosine and depressed tyrosine/NAA

ratios among low protein-fed cotton rats reflected lower concentrations of tyrosine, probably in response to lower enzyme activity. Young and Marchini (1990) also found that the phenylalanine/tyrosine ratio is a sensitive parameter that is elevated for animals under protein malnutrition.

Both rate of protein synthesis and muscle catabolism change as an animal grows and develops (Armstrong and Stave, 1973; Pastor-Anglada et al., 1987). Because diet-sample statistical interactions were significant for only tryptophan and hydroxyproline, observed temporal changes most likely reflected normal age-related alterations in physiology due to development and not to effects of diet. Younger rodents typically conserve EAA more efficiently in the muscle tissue, which is the main store of protein and free amino acids (Sketcher and James, 1974), and results in lower serum concentrations. Similarly, concentrations of the EAA histidine, tryptophan, lysine, and phenylalanine were lower at 30 days compared to 45 days. Older animals are known to convert carbohydrates to NEAA more efficiently than younger animals (Sketcher and James, 1974), which may have accounted for the observed elevations in concentrations of citrulline, cysteine, and ornithine in the cotton rat at 45 days compared to 30 days.

As with humans and laboratory animals, this study suggests that serum amino acid profiles can provide a sensitive measure of protein nutritional status in the cotton rat and can probably be adapted for use in other

species of small mammal. Because temporal alterations of serum free amino acid concentrations occur due to alterations in physiology associated with development, care must be taken when using serum free amino acids to ascertain protein nutritional status in juvenile animals. Several measures were insensitive to developmental alterations and should provide a more useful index for assessing protein nutritional status in wild animals. In particular, the free amino acids threonine, hydroxyproline, and serine, SAA, and the glycine/(leucine + valine) ratio appeared to be the most useful measurements in this study.

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Table 1.--Ingredient composition of isocaloric experimental diets containing either 4 or 16% protein. Diets were prepared by United States Biochemical, Cleveland, OH.

Ingredient	Percent air-dry basis	
	4%	16%
High nitrogen casein	4.00	16.00
Corn starch	82.00	70.00
Cotton seed oil	10.00	10.00
Salt mixture USP XIV	4.00	4.00

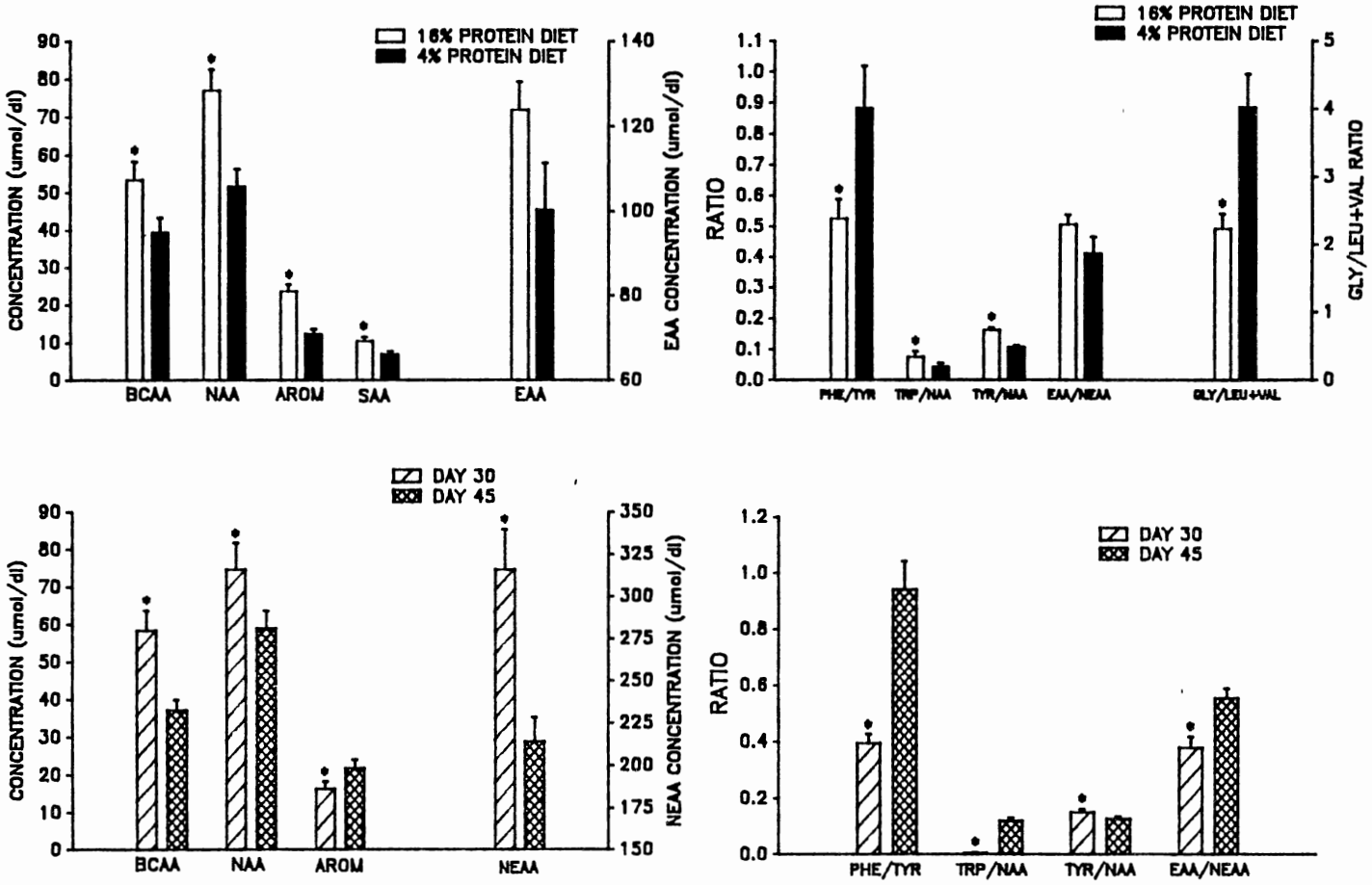
Table 2.--Mean concentrations (± SE) (μmol/dl) of essential and non-essential amino acids in blood serum of cotton rats fed either a 16% or 4% protein diet for 30 or 45 days.

	30-Day Sample		45-Day Sample		ANOVA(P)	
	16% Protein	4% Protein	16% Protein	4% Protein	Diet[a]	Sample[b]
Amino acid	(n = 9)	(n = 6)	(n = 9)	(n = 6)		
Essential amino acids						
Histidine	4.44 ± 0.45	5.71 ± 1.27	7.12 ± 0.56	12.69 ± 2.31	0.006	0.004
Isoleucine	28.06 ± 4.73	23.47 ± 2.57	13.23 ± 1.10	13.46 ± 1.99	0.512	0.001
Leucine	14.13 ± 2.36	8.83 ± 1.50	10.67 ± 1.40	8.80 ± 1.90	0.077	0.305
Lysine	23.54 ± 4.55	20.25 ± 5.73	40.92 ± 2.06	40.01 ± 9.39	0.699	0.002
Methionine	8.91 ± 1.24	5.17 ± 0.69	4.30 ± 0.33	3.03 ± 0.38	0.007	0.001
Phenylalanine	4.68 ± 0.41	3.07 ± 0.28	6.99 ± 0.87	6.09 ± 1.19	0.117	0.003
Threonine	17.74 ± 2.35	10.27 ± 1.15	12.37 ± 0.99	11.33 ± 2.00	0.028	0.118
Tryptophan	0.09 ± 0.04	0.63 ± 0.25	9.85 ± 0.74	3.46 ± 0.56	0.001	0.001
Valine	25.12 ± 2.84	12.57 ± 2.55	15.51 ± 1.81	11.66 ± 2.69	0.003	0.038
Non-essential amino acids						
Alanine	105.10 ± 12.76	102.94 ± 16.15	43.28 ± 5.72	37.93 ± 7.24	0.740	0.001
Asparagine	30.35 ± 3.42	35.26 ± 5.14	37.29 ± 3.50	32.59 ± 2.33	0.171	0.392
Aspartic acid	2.14 ± 0.43	1.87 ± 0.33	1.46 ± 0.33	1.99 ± 0.29	0.744	0.315
Citrulline	8.96 ± 0.76	8.75 ± 0.65	11.59 ± 0.90	14.30 ± 1.32	0.194	0.001
Cysteine	1.50 ± 1.03	1.08 ± 0.36	6.18 ± 0.81	4.56 ± 1.27	0.300	0.001
Glutamic acid	14.18 ± 1.24	14.29 ± 1.21	12.88 ± 1.92	17.92 ± 2.64	0.172	0.677
Glycine	64.78 ± 7.19	72.97 ± 10.62	66.22 ± 6.43	72.32 ± 1.25	0.414	0.942
Hydroxyproline	3.28 ± 0.23	3.18 ± 0.66	4.15 ± 0.36	2.32 ± 0.55	0.035	0.688
1-Methylhistidine	0.02 ± 0.01	0.13 ± 0.13	trace	trace	0.296	0.248
3-Methylhistidine	0.07 ± 0.04	0.12 ± 0.12	trace	trace	0.656	0.114
Ornithine	3.02 ± 0.63	2.67 ± 0.30	7.88 ± 0.83	9.30 ± 2.28	0.635	0.001
Proline	35.02 ± 4.86	69.29 ± 21.97	15.62 ± 1.82	18.52 ± 3.14	0.060	0.007
Serine	38.19 ± 3.61	28.45 ± 2.37	29.57 ± 2.33	24.25 ± 1.72	0.015	0.454
Taurine	13.67 ± 1.93	23.13 ± 6.26	13.74 ± 2.28	13.66 ± 0.86	0.155	0.189
Tyrosine	15.61 ± 2.11	6.53 ± 1.03	9.97 ± 1.19	4.72 ± 0.80	0.001	0.019

[a] 30- and 45-day data sets were combined.

[b] 4 and 16% data sets were combined within each sample time.

Figure 1. Effect of dietary protein (16 or 4%) and duration of feeding trial on selected free amino acid concentrations and amino acid ratios (BCAA = branch chain amino acids, NAA = neutral amino acids, AROM = aromatic amino acids, SAA = sulfur-containing amino acids, EAA = essential amino acids, NEAA = non-essential amino acids, PHE/TYR = phenylalanine/tyrosine, TRP/NAA = tryptophan/neutral amino acids, TYR/NAA = tyrosine/neutral amino acids, GLY/LEU+VAL = glycine/(leucine + valine)) in serum of juvenile cotton rats (* = significant difference at $\underline{P} \leq 0.05$).


16% PROTEIN DIET
4% PROTEIN DIET
CONCENTRATION (umol/dl)
EAA CONCENTRATION (umol/dl)
BCAA
NAA
AROM
SAA
EAA
RATIO
GLY/LEU+VAL RATIO
PHE/TYR
TRP/NAA
TYR/NAA
EAA/NEAA
GLY/LEU+VAL
DAY 30
DAY 45
NEAA CONCENTRATION (umol/dl)
NEAA

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